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(54) Title: HYPERSENSITIVE RESPONSE ELICITOR-I (57) Abstract	INDUC	ED STRESS RESISTANCE								

The present invention is directed to imparting stress resistance to plants. This can be achieved by applying a hypersensitive response elicitor in a non-infectious form to plants or plant seeds under conditions effective to impart stress resistance to plants or plants grown from the plant seeds. Alternatively, transgenic plants or plant seeds transformed with a DNA molecule encoding the elicitor can be provided and the transgenic plants or plants resulting from the transgenic plant seeds are grown under conditions effective to impart stress resistance to plants or plants grown from the plant seeds.

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HYPERSENSITIVE RESPONSE ELICITOR-INDUCED STRESS RESISTANCE

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FIELD OF THE INVENTION

The present invention relates to imparting stress resistance to plants with a hypersensitive response elicitor.

BACKGROUND OF THE INVENTION

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Under both natural and agricultural conditions, plants are exposed to various forms of environmental stress. Stress is mainly measured with respect to growth (i.e. biomass accumulation) or with respect to the primary assimilation processes (i.e. carbon dioxide and mineral intake). Soil water deficits, suboptimal and supraoptimal temperatures, salinity, and poor aeration of soils may each cause some growth restrictions during the growing season, so that the yield of plants at the end of the season expresses only a small fraction of their genetic potential. Indeed, it is estimated that in the United States the yield of field-grown crops is only 22% of genetic potential. The same physicochemical factors can become extreme in some habitats, such as deserts or marshes, and only specially adapted vegetation can complete its life cycle in the unusually hostile conditions. In less extreme environments, individual plants can become acclimated to changes in water potential, temperature, salinity, and oxygen deficiency so that their fitness for those environments improves. Some species are better able to adapt than others, and various anatomical, structural, and biochemical mechanisms account for acclimation.

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Under natural and agriculture conditions, plants must constantly endure stress. Some environmental factors can become stressful in a very short period of time (e.g., high or low temperature) or may take long periods of time to stress plants (e.g., soil water content or mineral nutrients). Generally, environmental stress effecting plants can be in the form of climate related stress, air pollution stress,

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chemical stress, and nutritional stress. Examples of climate related stress include drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light. Air pollution stress can be in the form of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain. Chemical stress can result from application of insecticides, fungicides, herbicides, and heavy metals. Nutritional stress can be caused by fertilizers, micronutrients, and macronutrients.

For most plants, water is essential for growth. Some plants are able to preserve some water in the soil for later use, while others complete their life cycles during a wet season before the onset of any drought. Other plants are able to aggressively consume water to save themselves while causing water deprivation for other plants in that location. Plants lacking any of these capabilities are severely hampered by the absence of water.

Chilling injury occurs in sensitive species at temperatures that are too low for normal growth but not sufficiently low to form ice. Such injury typically occurs in species of tropical or subtropical origin. When chilling occurs, discoloration or lesions appear on leaves giving them a water-soaked appearance. If roots are chilled, the plants may wilt. On the other hand, freezing temperatures and the accompanying formation of ice crystals in plants can be lethal if ice crystals extend into protoplasts or remain for long periods.

Stress is also caused by the other temperature extremes with few plants being able to survive high temperatures. When higher plant cells or tissues are dehydrated or are not growing, they can survive higher temperatures than cells which are hydrated, vegetative, and growing. Tissues which are actively growing can rarely survive at temperatures above 45°C.

High salt concentrations are another form of environmental stress which can afflict plants. In natural conditions, such high concentrations of salt are found close to seashores and estuaries. Farther inland, natural salt may seep from geological deposits adjoining agricultural areas. In addition, salt can accumulate in irrigation water when pure water is evaporated or transpired from soil. About 1/3 of all irrigated farmland is effected by high salt concentrations. High salt content not

only injures plants but degrades soil structure by decreasing porosity and water permeability.

Air pollution in the form of ozone, carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, and hydrocarbons can very adversely effect plant growth by creating smog and environmental warming.

The present invention is directed to overcoming various forms of environmental stress and imparting resistance in plants to such stress.

SUMMARY OF THE INVENTION

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The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

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Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air polllution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Applicants have found that use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

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DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of a hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants. In one

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embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide is applied to plants or plant seeds under conditions effective to impart stress resistance. Alternatively, the stress resistance is imparted by providing a transgenic plant or plant seed transformed with a DNA molecule which encodes for a hypersensitive response elicitor protein or polypeptide and growing the transgenic plant or plants produced from the transgenic plant seeds under conditions effective to impart stress resistance.

The hypersensitive response elicitor polypeptides or proteins according to the present invention are derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors include Erwinia, Pseudomonas, and Xanthamonas species (e.g., the following bacteria: Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is Clavibacter michiganensis subsp. sepedonicus.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica*, *Phytophthora cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from Erwinia chrysanthemi has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser 10

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser 30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr Asp Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu Ser 55

*	Gl y 65	Ala	Ser	Ser	Lys	Gly 70	Leu	Gly	Met	Ser	Asn 75	Gln	Leu	Gly	Gln	Ser 80
	Phe	Gly	Asn	Gly	Ala 85	Gln	Gly	Ala	Ser	Asn 90	Leu	Leu	Ser	Val	Pro 95	Lys
5		_		100					105					110	Asp	
			115					120					125		Asn	
10		130					135					140			naA	
	145					150					155				Leu	160
					165					170					Leu 175	
15				180					185					190	Gln	
			195					200					205		Ser	
20		210					215					220			Phe	
	225					230					235				Met	240
					245					250					Gly 255 Ser	
25				260					265					270	Arg	
			275	•				280					285	•		Thr
30		290)				295	•				300	,			Ala
	305	;				310)				315	5				320 Ala
				. GI	32!					330)			- 4	335	i
35	ASI	a Ale	a													

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains

substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

5	CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG	60
	GOSTITATES COGCEATGAN COGGEATCAG GOGGEGGCT GGTCGCCGCA ATCCGGCGTC	120
	GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG	180
	CAGCAATATC COGGCATGTT GOGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG	240
	TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG	300
10	CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG	360
	ACGITGCCGI CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC	420
	CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT	480
	CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG	540
	GGCATCOGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA	600
15	AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC	660
•	TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT	720
	GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT	780
	GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC	840
	TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA	900
20	TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC	960
•	CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC	1020
	CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG	1080
	CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT	1140
	GCAGGGCCTG AGCGGCGCG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT	1200
25	GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA	1260
	CCGCCACTIT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA	132
	TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA	138
•	GACGGACGAC ARATCCTGGG CTARAGCGCT GAGTARACCG GATGATGACG GTATGACCGG	144
	OGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA	150
30	TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC	156
	COCCATABAR TRECCARCAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA	162

	ATCTGTGCTG	GCCTGATAAA	GCGGAAACGA	AAAAAGAGAC	GGGGAAGCCT	GTCTCTTTTC	1680
	TTATTATGCG	GTTTATGCGG	TTACCTGGAC	CGGTTAATCA	TCGTCATCGA	TCTGGTACAA	1740
	ACGCACATTT	TCCCGTTCAT	TOGOGTOGTT	ACGCCCACA	ATCGCGATGG	CATCTTCCTC	1800
	GTCGCTCAGA	TTGCGCGGCT	GATGGGGAAC	GCCGGGTGGA	ATATAGAGAA	ACTOGCOGGO	1860
;	CAGATGGAGA	CACGTCTGCG	ATAAATCTGT	GCCGTAACGT	GTTTCTATCC	GCCCCTTTAG	1920
	CAGATAGATT	GCGGTTTCGT	AATCAACATG	GTAATGCGGT	TCCGCCTGTG	CGCCGGCCGG	1980
	GATCACCACA	ATATTCATAG	AAAGCTGTCT	TGCACCTACC	GTATCGCGGG	AGATACCGAC	2040
	AAAATAGGGC	AGTTTTTGCG	TGGTATCCGT	GGGTGTTCC	GGCCTGACAA	TCTTGAGTTG	2100
	GTTCGTCATC	ATCTTTCTCC	ATCTGGGCGA	CCTGATCGGT	T		2141

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The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

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| Met 1 | Ser | Leu | Asn | Thr | Ser | Gly | Leu | Gly | Ala | Ser | Thr | Met | Gln | Ile | Ser | Ile | Gly | Gly | Ala | Gly | Gly | Asn | Asn | Gly | Leu | Leu | Gly | Thr | Ser | Arg | Gln | 30 | Ser | Asn | Ala | Gly | Leu | Gly | Gly | Asn | Ser | Ala | Leu | Gly | Leu | Gly | Gly | Asn | Ser | Asn | Gln | Asn | Asn | Ser | Asn | Gln | Leu | Ala | Gly | Leu | Gly | Gly | Asn | Ser |

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln 35 145 150 155 160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly 165 170 175

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly 195 200 205 Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly 5 Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln 10 Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met 15 Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser 20 Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn Gly Asn Leu Glm Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp 25 Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu Gly Ala Ala

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*,"

Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

5	AAGCTTCGGC	ATGGCACGTT	TGACCGTTGG	GTCGGCAGGG	TACGTTTGAA	TTATTCATAA	60
	GAGGAATACG	TTATGAGTCT	GAATACAAGT	GGGCTGGGAG	CETCAACGAT	GCAAATTTCT	120
	ATCGGCGGTG	CGGGCGGAAA	TAACGGGTTG	CTGGGTACCA	GTCGCCAGAA	TGCTGGGTTG	180
	GGTGGCAATT	CTGCACTGGG	CTGGGCGGC	GGTAATCAAA	ATGATACCGT	CAATCAGCTG	240
	GCTGGCTTAC	TCACCGGCAT	GATGATGATG	ATGAGCATGA	TGGGCGGTGG	TGGGCTGATG	300
10	GGCGGTGGCT	TAGGCGGTGG	CTTAGGTAAT	GCCTTGGGTG	GCTCAGGTGG	CCTGGGCGAA	360
	GGACTGTCGA	ACGCGCTGAA	CGATATGTTA	GCCGGTTCGC	TGAACACGCT	GGGCTCGAAA	420
	GGCGGCAACA	ATACCACTTC	AACAACAAAT	TCCCCGCTGG	ACCAGGCGCT	GGGTATTAAC	480
	TCAACGTCCC	AAAACGACGA	TTCCACCTCC	GGCACAGATT	CCACCTCAGA	CTCCAGCGAC	540
	CCGATGCAGC	AGCTGCTGAA	GATGTTCAGC	GAGATAATGC	AAAGCCTGTT	TGGTGATGGG	600
15	CAAGATGGCA	CCCAGGGCAG	TTCCTCTGGG	GGCAAGCAGC	CGACCGAAGG	CGAGCAGAAC	660
	GCCTATAAAA	AAGGAGTCAC	TGATGCGCTG	TCGGGCCTGA	TGGGTAATGG	TCTGAGCCAG	720
	CTCCTTGGCA	ACGGGGGACT	GGGAGGTGGT	CAGGGCGGTA	ATGCTGGCAC	GGGTCTTGAC	780
	GGTTCGTCGC	TGGGCGGCAA	AGGGCTGCAA	AACCTGAGCG	GGCCGGTGGA	CTACCAGCAG	840
	TTAGGTAACG	CCGTGGGTAC	CGGTATCGGT	ATGAAAGCGG	GCATTCAGGC	GCTGAATGAT	900
20	ATCGGTACGC	ACAGGCACAG	TTCAACCCGT	TCTTTCGTCA	ATAAAGGCGA	TCGGGCGATG	960
	GCGAAGGAAA	TOGGTCAGTT	CATGGACCAG	TATCCTGAGG	TGTTTGGCAA	GCCGCAGTAC	1020
	CAGAAAGGCC	CGGGTCAGGA	GGTGAAAACC	GATGACAAAT	CATGGGCAAA	AGCACTGAGC	1080
	AAGCCAGATG	ACGACGGAAT	GACACCAGCC	AGTATGGAGC	AGTTCAACAA	AGCCAAGGGC	1140
	ATGATCAAAA	GGCCCATGGC	GGGTGATACC	GGCAACGGCA	ACCTGCAGGC	ACGCGGTGCC	1200
25	GGTGGTTCTT	CGCTGGGTAT	TGATGCCATG	ATGGCCGGTG	ATGCCATTAA	CAATATGGCA	1260
	CTTGGCAAGC	TGGGCGCGGC	TTAAGCTT				1288

Another potentially suitable hypersensitive response elicitor from

Erwinia amylovora is disclosed in U.S. Patent Application Serial No. 09/120,927,
which is hereby incorporated by reference. The protein is encoded by a DNA
molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

	ATGTCAATTC	TTACGCTTAA	CAACAATACC	TOGTOCTOGO	CGGGTCTGTT	CCAGTCCGGG	60
	GGGGACAACG	GGCTTGGTGG	TCATAATGCA	AATTCTGCGT	TGGGGCAACA	ACCCATCGAT	120
5	CGGCAAACCA	TTGAGCAAAT	GGCTCAATTA	TTGGCGGAAC	TGTTAAAGTC	ACTGCTATCG	180
	CCACAATCAG	GTAATGCGGC	AACCGGAGCC	GGTGGCAATG	ACCAGACTAC	AGGAGTTGGT	240
10	AACGCTGGCG	GCCTGAACGG	ACGAAAAGGC	ACAGCAGGAA	CCACTCCGCA	GTCTGACAGT	300
	CAGAACATGC	TGAGTGAGAT	GGGCAACAAC	GGGCTGGATC	AGGCCATCAC	GCCCGATGGC	360
	CAGGGCGGCG	GGCAGATCGG	CGATAATCCT	TTACTGAAAG	CCATGCTGAA	GCTTATTGCA	420
15	CGCATGATGG	ACGGCCAAAG	CGATCAGTTT	GGCCAACCTG	GTACGGGCAA	CAACAGTGCC	480
	TCTTCCGGTA	CTTCTTCATC	TGGCGGTTCC	CCTTTTAACG	ATCTATCAGG	GGGGAAGGCC	540
20	CCTTCCGGCA	ACTCCCCTTC	CGGCAACTAC	TCTCCCGTCA	GTACCTTCTC	ACCCCCATCC	600
	ACGCCAACGT	CCCCTACCTC	ACCGCTTGAT	TTCCCTTCTT	CTCCCACCAA	AGCAGCOGGG	660
	GGCAGCACGC	CGGTAACCGA	TCATCCTGAC	CCTGTTGGTA	GCGCGGGCAT	CGGGGCCGGA	720
25	AATTCGGTGG	CCTTCACCAG	CGCCGGCGCT	AATCAGACGG	TGCTGCATGA	CACCATTACC	780
	GTGAAAGCGG	GTCAGGTGTT	TGATGGCAAA	GGACAAACCT	TCACCGCCGG	TTCAGAATTA	840
30	GGCGATGGCG	GCCAGTCTGA	AAACCAGAAA	CCGCTGTTTA	TACTGGAAGA	CGGTGCCAGC	900
	CTGAAAAACG	TCACCATGGG	CGACGACGGG	GCGGATGGTA	TTCATCTTTA	CGGTGATGCC	960
	AAAATAGACA	ATCTGCACGT	CACCAACGTG	GGTGAGGACG	CGATTACCGT	TAAGCCAAAC	1020
35	AGCGCGGGCA	AAAAATCCCA	CGTTGAAATC	ACTAACAGTT	CCTTCGAGCA	CGCCTCTGAC	1080
	AAGATCCTGC	AGCTGAATGC	CGATACTAAC	CTGAGCGTTG	ACAACGTGAA	GGCCAAAGAC	1140
40	TTTGGTACTT	TTGTACGCAC	TAACGGCGGT	CAACAGGGTA	ACTGGGATCT	GAATCTGAGC	1200
	CATATCAGCG	CAGAAGACGG	TAAGTTCTCG	TICGTTAAAA	GCGATAGCGA	GGGGCTAAAC	1260
	GTCAATACCA	GTGATATCTC	ACTGGGTGAT	GTTGAAAACC	ACTACAAAGI	GCCGATGTCC	1320
45	GCCAACCTGA	AGGTGGCTGA	ATGA				1344

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 6 as follows:

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Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu 15

Phe Gln Ser Gly Gly Asp Asn Gly Leu 25

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala 60

	Gln	Leu 50	Leu	Ala	GIU	Leu	Leu 55	Lys	ser	Leu	ren	60	PIO	GIN	ser	СІУ
5	Asn 65	Ala	Ala	Thr	Gly	Ala 70	Gly	Gly	Asn	Asp	Gln 75	Thr	Thr	Gly	Val	Gly 80
	Asn	Ala	Gly	Gly	Leu 85	Asn	Gly	Arg	Lys	Gly 90	Thr	Ala	Gly	Thr	Thr 95	Pro
10	Gln	ser	Asp	Ser 100	Gln	asA	Met	Leu	Ser 105	Glu	Met	Gly	Asn	Asn 110	Gly	Leu
15	Asp	Gln	Ala 115	Ile	Thr	Pro	Asp	Gly 120	Gln	Gly	Gly	Gly	Gln 125	Ile	Gly	Asp
	Asn	Pro 130	Leu	Leu	Lys	Ala	Met 135	Leu	Lys	Leu	Ile	Ala 140	Arg	Met	Met	Asp
20	Gly 145	Gln	Ser	Asp	Gln	Phe 150	Gly	Gln	Pro	Gly	Thr 155	Gly	Asn	Asn	Ser	Ala 160
25	Ser	Ser	Gly	Thr	Ser 165	Ser	Ser	Gly	Gly	Ser 170	Pro	Phe	Asn	ДБР	Leu 175	Ser
23	Gly	Gly	Lys	Ala 180	Pro	Ser	Gly	Asn	Ser 185	Pro	Ser	Gly	Asn	Тут 190	Ser	Pro
30	Val	Ser	Thr 195	Phe	Ser	Pro	Pro	Ser 200	Thr	Pro	Thr	Ser	Pro 205	Thr	Ser	Pro
	Leu	Asp 210	Phe	Pro	Ser	Ser	Pro 215	Thr	Lys	Ala	Ala	Gly 220	Gly	Ser	Thr	Pro
35	Val 225	Thr	Asp	His	Pro	Asp 230	Pro	Val	Gly	Ser	Ala 235	Gly	lle	Gly	Ala	Gly 240
40	Asn	Ser	Val	Ala	Phe 245	Thr	Ser	Ala	Gly	Ala 250	Asn	Gln	Thr	Val	Leu 255	His
10	Asp	Thr	Ile	Thr 260	Val	Lys	Ala	Gly	Gln 265	Val	Phe	qeA	Gly	Lys 270	Gly	Gln
45	Thr	Phe	Thr 275	Ala	Gly	Ser	Glu	Leu 280	Gly	Asp	Gly	Gly	Gln 285	Ser	Glu	Asn
	Gln	Lys 290	Pro	Leu	Phe	Ile	Leu 295	Glu	qaA	Gly	Ala	Ser 300	Leu	Lys	Asn	Val
50	Thr 305	Met	Gly	Asp	qeA	Gly 310	Ala	Asp	Gly	Ile	His 315	Leu	Tyr	Gly	Asp	Ala 320
55	Lys	Ile	Asp	Asn	Leu 325	His	Val	Thr	Asn	Val 330	Gly	Glu	Asp	Ala	Ile 335	Thr
,,	Val	Lys	Pro	Asn 340	Ser	Ala	Gly	Lys	Lys 345	Ser	His	Val	Glu	11e 350	Thr	Asn

	Ser	Ser	Phe 355	Glu	His	Ala	Ser	Авр 360	Lys	Ile	Leu	Gln	Leu 365	Asn	Ala	As p
5	Thr	Asn 370	Leu	Ser	Val	Asp	Asn 375	Val	Lys	Ala	Lys	Asp 380	Phe	Gly	Thr	Phe
	Val 385	Arg	Thr	Asn	Gly	390 Gly	Gln	Gln	Gly	Asn	Trp 395	Asp	Leu	Asn	Leu	Ser 400
10	His	Ile	Ser	Ala	Glu 405	Asp	Gly	Lys	Phe	Ser 410	Phe	Val	Lys	Ser	Asp 415	Ser
	Glu	Gly	Leu	Asn 420	Val	Asn	Thr	Ser	Asp 425	Ile	Ser	Leu	Gly	Asp 430	Val	Glu
15	Asn	His	Tyr 435	Lys	Val	Pro	Met	Ser 440	Ala	Asn	Leu	Lys	Val 445	Ala	Glu	
							• .	. •	1		:		-d lo	oleo o		. I

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

Another potentially suitable hypersensitive response elicitor from

Erwinia amylovora is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

	ATGGAATTAA	AATCACTGGG	AACTGAACAC	AAGGCGGCAG	TACACACAGC	GGCGCACAAC	60
30	CCTGTGGGGC	ATGGTGTTGC	CTTACAGCAG	GGCAGCAGCA	GCAGCAGCCC	GCAAAATGCC	120
	GCTGCATCAT	TGGCGGCAGA	AGGCAAAAAT	CGTGGGAAAA	TGCCGAGAAT	TCACCAGCCA	180
35	TCTACTGCGG	CTGATGGTAT	CAGCGCTGCT	CACCAGCAAA	AGAAATCCTT	CAGTCTCAGG	240
	GGCTGTTTGG	GGACGAAAA	ATTTTCCAGA	TCGGCACCGC	AGGGCCAGCC	AGGTACCACC	300
40	CACAGCAAAG	GGGCAACATT	GCGCGATCTG	CTGGCGCGGG	ACGACGGCGA	AACGCAGCAT	360
40	GAGGCGGCCG	CGCCAGATGC	GGCGCGTTTG	ACCCGTTCGG	GCGGCGTCAA	ACGCCGCAAT	420
	ATGGACGACA	TGGCCGGGCG	GCCAATGGTG	AAAGGTGGCA	GCGGCGAAGA	TAAGGTACCA	480
45	ACGCAGCAAA	AACGGCATCA	GCTGAACAAT	TTTGGCCAGA	TGCGCCAAAC	GATGTTGAGC	540
	AAAATGGCTC	ACCCGGCTTC	AGCCAACGCC	GGCGATCGCC	TGCAGCATTC	ACCGCCGCAC	600
	ATCCCGGGTA	GCCACCACGA	AATCAAGGAA	GAACCGGTTG	GCTCCACCAG	CAAGGCAACA	660
50	ACGGCCCACG	CAGACAGAGT	GGAAATCGCT	CAGGAAGATG	ACGACAGCGA	ATTCCAGCAA	720
	CTGCATCAAC	AGCGGCTGGC	GOGCGAACGG	GAAAATCCAC	CGCAGCCGCC	CAAACTCGGC	780
55	GTTGCCACAC	CGATTAGCGC	CAGGTTTCAG	CCCAAACTGA	CTGCGGTTGC	GGAAAGCGTC	840

	CTTGAGGGGA	CAGATACCAC	GCAGTCACCC	CTTARGCCGC	AATCAATGCT	GAAAGGAAGT	900
	GGAGCCGGGG	TAACGCCGCT	GGCGGTAACG	CTGGATAAAG	GCAAGTTGCA	GCTGGCACCG	960
5	GATAATCCAC	CCGCGCTCAA	TACGTIGITG	AAGCAGACAT	TGGGTAAAGA	CACCCAGCAC	1020
	TATCTGGCGC	ACCATGCCAG	CAGCGACGGT	AGCCAGCATC	TGCTGCTGGA	CAACAAAGGC	1080
10	CACCTGTTTG	ATATCAAAAG	CACCGCCACC	AGCTATAGCG	TGCTGCACAA	CAGCCACCCC	1140
	GGTGAGATAA	AGGGCAAGCT	GGCGCAGGCG	GGTACTGGCT	CCGTCAGCGT	AGACGGTAAA	1200
	AGCGGCAAGA	TCTCGCTGGG	GAGCGGTACG	CAAAGTCACA	ACAAAACAAT	GCTAAGCCAA	1260
15	CCGGGGGAAG	OGCACOGTTC	CTTATTAACC	GGCATTTGGC	AGCATCCTGC	TGGCGCAGCG	1320
	CGGCCGCAGG	GCGAGTCAAT	COGCCTGCAT	GACGACAAAA	TTCATATCCT	GCATCCGGAG	1380
20	CTGGGCGTAT	GGCAATCTGC	GGATAAAGAT	ACCCACAGCC	AGCTGTCTCG	CCAGGCAGAC	1440
	GGTAAGCTCT	ATGCGCTGAA	AGACAACCGT	ACCCTGCAAA	ACCTCTCCGA	TAATAAATCC	1500
25	TCAGAAAAGC	TGGTCGATAA	AATCAAATCG	TATTCCGTTG	ATCAGCGGGG	GCAGGTGGCG	1560
25	ATCCTGACGG	ATACTCCCGG	CCGCCATAAG	ATGAGTATTA	TGCCCTCGCT	GGATGCTTCC	1620
-	CCGGAGAGCC	ATATTTCCCT	CAGCCTGCAT	TTTGCCGATG	CCCACCAGGG	GTTATTGCAC	1680
30	GGGAAGTCGG	AGCTTGAGGC	ACAATCTGTC	GCGATCAGCC	ATGGGCGACT	GGTTGTGGCC	1740
	GATAGCGAAG	GCAAGCTGTT	TAGCGCCGCC	ATTCOGAAGC	AAGGGGATGG	AAACGAACTG	1800
35	AAAATGAAAG	CCATGCCTCA	GCATGCGCTC	GATGAACATT	TTGGTCATGA	CCACCAGATT	1860
33						TAACTTCAGG	1920
	CAGCAGCATG	CCTGCCCGTT	GGGTAACGAT	CATCAGTTTC	ACCCCGGCTG	GAACCTGACT	1980
40	GATGCGCTGG	TTATCGACAA	TCAGCTGGGG	CTGCATCATA	CCAATCCTGA	ACCGCATGAG	2040
	ATTCTTGATA	TGGGGCATTT	AGGCAGCCTG	GCGTTACAGG	AGGGCAAGCT	TCACTATTTT	2100
45						GAAAAAAGGC	2160
45	CTGGATGGAG	CAGCTTATCT	ACTGAAAGAC	GGTGAAGTGA	AACGCCTGAA	TATTAATCAG	2220
	AGCACCTCCT	CTATCAAGCA	CGGAACGGAA	AACGTTTTTT	CGCTGCCGCA	TGTGCGCAAT	2280
50	AAACCGGAGC	CGGGAGATGC	CCTGCAAGGG	CTGAATAAAG	ACGATAAGGC	CCAGGCCATG	2340
						TOGCTCCTTC	2400
55	CAGATAAAAC	CCGGCACCCA	GCAGTTGGAG	CGGCCGGCAC	AAACTCTCAG	CCGCGAAGGT	2460
<i></i>	ATCAGCGGCG	AACTGAAAGA	CATTCATGTC	GACCACAAGC	AGAACCTGTA	TGCCTTGACC	2520
						CGAAAGCAGC	2580
60						GGACATGAGC	2640
						GAAGGCTGGC	2700
65	GGCTGGCACG	CCTATGCGGC	ACCTGAACGC	GGGCCGCTGG	CGGTGGGTAC	CAGCGGTTCA	2760

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	CAAACCGTCT	TTAACCGACT	aatgcaggg	GTGAAAGGCA	AGGTGATCCC	AGGCAGCGGG	2820
	TTGACGGTTA	AGCTCTCGGC	TCAGACGGGG	GGAATGACCG	GCGCCGAAGG	GCGCAAGGTC	2880
5	AGCAGTAAAT	TTTCCGAAAG	GATCCGCGCC	TATGCGTTCA	ACCCAACAAT	GTCCACGCCG	2940
	CGACCGATTA	AAAATGCTGC	TTATGCCACA	CAGCACGGCT	GGCAGGGGCG	TGAGGGGTTG	3000
	AAGCCGTTGT	ACGAGATGCA	GGGAGCGCTG	ATTAAACAAC	TGGATGCGCA	TAACGTTCGT	3060
10	CATAACGCGC	CACAGCCAGA	TTTGCAGAGC	AAACTGGAAA	CTCTGGATTT	AGGCGAACAT	3120
	GGCGCAGAAT	TGCTTAACGA	CATGAAGCGC	TTCCGCGACG	aactggagca	GAGTGCAACC	3180
15	CGTTCGGTGA	CCGTTTTAGG	TCAACATCAG	GGAGTGCTAA	AAAGCAACGG	TGAAATCAAT	3240
	AGCGAATTTA	AGCCATCGCC	CGGCAAGGCG	TTGGTCCAGA	GCTTTAACGT	CAATOGCTCT	3300
	GGTCAGGATC	TAAGCAAGTC	ACTGCAACAG	GCAGTACATG	CCACGCCGCC	ATCCGCAGAG	3360
20	AGTAAACTGC	AATCCATGCT	GGGGCACTTT	GTCAGTGCCG	GGGTGGATAT	GAGTCATCAG	3420
	AAGGGCGAGA	TCCCGCTGGG	CCGCCAGCGC	GATCCGAATG	ATAAAACCGC	ACTGACCAAA	3480
25	TCGCGTTTAA	TTTTAGATAC	CGTGACCATC	GGTGAACTGC	ATGAACTGGC	CGATAAGGCG	3540
	AAACTGGTAT	CTGACCATAA	ACCCGATGCC	GATCAGATAA	AACAGCTGCG	CCAGCAGTTC	3600
30	GATACGCTGC	GTGAAAAGCG	GTATGAGAGC	AATCCGGTGA	AGCATTACAC	CGATATGGGC	3660
30	TTCACCCATA	ATAAGGCGCT	GGAAGCAAAC	TATGATGCGG	TCAAAGCCTT	TATCAATGCC	3720
	TTTAAGAAAG	AGCACCACGG	CGTCAATCTG	ACCACGCGTA	CCGTACTGGA	ATCACAGGGC	3780
35	AGTGCGGAGC	TGGCGAAGAA	GCTCAAGAAT	ACCCTGTTGT	CCCTGGACAG	TGGTGAAAGT	3840
	ATGAGCTTCA	GCCGGTCATA	TGGCGGGGGC	GTCAGCACTG	TCTTTGTGCC	TACCCTTAGC	3900
40	AAGAAGGTGC	CAGTTCCGGT	GATCCCCGGA	GCCGGCATCA	CGCTGGATCG	CGCCTATAAC	3960
40	CTGAGCTTCA	GTCGTACCAG	CGGCGGATTG	AACGTCAGTT	TTGGCCGCGA	CGGCGGGGTG	4020
	AGTGGTAACA	TCATGGTCGC	TACCGGCCAT	GATGTGATGC	CCTATATGAC	CGGTAAGAAA	4080
45	ACCAGTGCAG	GTAACGCCAG	TGACTGGTTG	AGCGCAAAAC	ATAAAATCAG	CCCGGACTTG	4140
	CGTATCGGCG	CTGCTGTGAG	TGGCACCCTG	CAAGGAACGC	TACAAAACAG	CCTGAAGTTT	4200
50	AAGCTGACAG	AGGATGAGCT	GCCTGGCTTT	ATCCATGGCT	TGACGCATGG	CACGTTGACC	4260
50	CCGGCAGAAC	TGTTGCAAAA	GGGGATCGAA	CATCAGATGA	AGCAGGGCAG	CAAACTGACG	4320
	TTTAGCGTCG	ATACCTCGGC	AAATCTGGAT	CTGCGTGCCG	GTATCAATCT	GAACGAAGAC	4380
55 -	GGCAGTAAAC	CAAATGGTGT	CACTGCCCGT	GTTTCTGCCG	GGCTAAGTGC	ATCGGCAAAC	4440
	CTGGCCGCCG	GCTCGCGTGA	ACGCAGCACC	ACCTCTGGCC	AGTTTGGCAG	CACGACTTCG	4500
60	GCCAGCAATA	ACCGCCCAAC	CTTCCTCAAC	GGGGTCGGCG	CGGGTGCTAA	CCTGACGGCT	4560
30	GCTTTAGGGG	TTGCCCATTC	ATCTACGCAT	GAAGGGAAAC	CGGTCGGGAT	CTTCCCGGCA	4620
	TTTACCTCGA	CCAATGTTTC	GGCAGCGCTG	GCGCTGGATA	ACCGTACCTC	ACAGAGTATC	4680
65	AGCCTGGAAT	TGAAGCGCGC	GGAGCCGGTG	ACCAGCAACG	ATATCAGCGA	GTTGACCTCC	4740

	ACGCTGGGAA	AACACTTTAA	GGATAGCGCC	ACAACGAAGA	TGCTTGCCGC	TCTCAAAGAG	4800
	TTAGATGACG	CTAAGCCCGC	TGAACAACTG	CATATTTTAC	AGCAGCATTT	CAGTGCAAAA	4860
5	GATGTCGTCG	GTGATGAACG	CTACGAGGCG	GTGCGCAACC	TGAAAAAACT	GGTGATACGT	4920
	CAACAGGCTG	CGGACAGCCA	CAGCATGGAA	TTAGGATCTG	CCAGTCACAG	CACGACCTAC	4980
10	AATAATCTGT	CGAGAATAAA	TAATGACGGC	ATTGTCGAGC	TGCTACACAA	ACATTTCGAT	5040
	GCGGCATTAC	CAGCAAGCAG	TGCCAAACGT	CTTGGTGAAA	TGATGAATAA	CGATCCGGCA	5100
1.5	CTGAAAGATA	TTATTAAGCA	GCTGCAAAGT	ACCCCTTCA	GCAGCGCCAG	CGTGTCGATG	5160
15	GAGCTGAAAG	ATGGTCTGCG	TGAGCAGACG	GAAAAAGCAA	TACTGGACGG	TAAGGTCGGT	5220
	CGTGAAGAAG	TGGGAGTACT	TTTCCAGGAT	CGTAACAACT	TGCGTGTTAA	ATCGGTCAGC	5280
20	GTCAGTCAGT	CCGTCAGCAA	AAGCGAAGGC	TTCAATACCC	CAGCGCTGTT	ACTGGGGACG	5340
	AGCAACAGCG	CTGCTATGAG	CATGGAGCGC	AACATCGGAA	CCATTAATTT	TAAATACGGC	5400
25	CAGGATCAGA	ACACCCCACG	GCGATTTACC	CTGGAGGGTG	GAATAGCTCA	GGCTAATCCG	5460
23	CAGGTCGCAT	CTGCGCTTAC	TGATTTGAAG	AAGGAAGGGC	TGGAAATGAA	GAGCTAA	5517

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated

DNA molecule of the present invention encodes a protein or polypeptide which elicits
a plant pathogen's hypersensitive response having an amino acid sequence of SEQ.

ID. No. 8 as follows:

35 Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr 15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser 25

40 Ser Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly 45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala Asp 65

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Lys Gly Ala Thr Leu Arg 80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln 95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala Ala Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Arg Asn Met Asp Asp Met 60

	Ala 145	Gly	Arg	Pro	Met	Val 150	Lys	Gly	Gly	Ser	Gly 155	Glu	Авр	Lys	Val	Pro 160
5	Thr	Gln	Gln	Lys	Arg 165	His	Gln	Leu	Asn	Asn 170	Phe	Gly	Gln	Met	Arg 175	Gln
	Thr	,Met	Leu	Ser 180	Lys	Met	Ala	His	Pro 185	Ala	Ser	Ala	Asn	Ala 190	Gly	Авр
10	Arg	Leu	Gln 195	His	Ser	Pro	Pro	His 200	Ile	Pro	Gly	Ser	His 205	His	Glu	Ile
15	Lys	Glu 210	Glu	Pro	Val	Gly	Ser 215	Thr	Ser	Lув	Ala	Thr 220	Thr	Ala	aiH	Ala
13	Asp 225	Arg	Val	Glu	Ile	Ala 230	Gln	Glu	qaA	Asp	Asp 235	Ser	Glu	Phe	Gln	Gln 240
20	Leu	His	Gln	Gln	Arg 245	Leu	Ala	Arg	Glu	Arg 250	Glu	Asn	Pro	Pro	Gln 255	Pro
			Leu	260					265					270		
25			Ala 275					280					285			
30		290	Leu				295					300				
	305		Leu			310					315					320
35	_		Pro		325					330					335	
			Gln	340					345					350		
40			Leu 355					360					365			
45		370	Ser				375					380				
	385		Leu			390					395					400
50			Lys		405					410					415	
			Ser	420					425					430		
55	Trp		435					440					445			
60		450	Asp		_		455					460				
	465		Ala			470					475					480
65	Gly	Lys	Leu	Tyr	Ala 485	Leu	Lys	Asp	Asn	Arg 490	Thr	Leu	Gln	Asn	Leu 495	Sei

	Asp	Asn	Lys	Ser 500	Ser	Glu	Lys	Leu	Val 505	Asp	Lys	Ile	Lys	Ser 510	Tyr	Ser
5	Val	Asp	Gln 515	Arg	Gly	Gln		Ala 520	Ile	Leu	Thr	Asp	Thr 525	Pro	Gly	Arg
	His	Lys 530	Met	Ser	Ile	Met	Pro 535	Ser	Leu	Asp	Ala	Ser 540	Pro	Glu	Ser	His
10	Ile 545	Ser	Leu	Ser	Leu	His 550	Phe	Ala	Asp	Ala	His 555	Gln	Gly	Leu	Leu	His 560
15	Gly	Lys	Ser	Glu	Leu 565	Glu	Ala	Gln	Ser	Val 570	Ala	Ile	Ser	His	Gly 575	Arg
13	Leu	Val	Val	Ala 580	Asp	Ser	Glu	Gly	Lys 585	Leu	Phe	Ser	Ala	Ala 590	Ile	Pro
20	Lys	Gln	Gly 595	Asp	Gly	Asn	Glu	Leu 600	Lys	Met	Lys	Ala	Met 605	Pro	Gln	His
		Leu 610	Asp	Glu	His	P'ne	Gly 615	His	Авр	His	Gln	Ile 620	Ser	Gly	Phe	Phe
25	His 625	Asp	Asp	His	Gly	Gln 630	Leu	Asn	Ala	Leu	Val 635	Lys	Asn	Asn	Phe	Arg 640
30	Gln	Gln	His	Ala	Сув 645	Pro	Leu	Gly	Asn	Asp 650	His	Gln	Phe	His	Pro 655	Gly
50	Trp	Asn	Leu	Thr 660	Asp	Ala	Leu	Val	11e 665	qeA	Asn	Gln	Leu	Gly 670	Leu	His
35	His	Thr	Asn 675	Pro	Glu	Pro	His	Glu 680	Ile	Leu	Asp	Met	Gly 685	His	Leu	Gly
		690	Ala				695					700				
40	Lys 705	Gly	Trp	Thr	Gly	Ala 710	Glu	Ser	Asp	Сув	Lys 715	Gln	Leu	Lys	Lys	Gly 720
45	Leu	Asp	Gly	Ala	Ala 725	Tyr	Leu	Leu	Lys	Asp 730	Gly	Glu	Val	Lys	Arg 735	Leu
-15	Asn	Ile	Asn	Gln 740	Ser	Thr	Ser	Ser	11e 745	Lys	His	Gly	Thr	Glu 750	Asn	Val
50	Phe	Ser	Leu 755	Pro	His	Val	Arg	Asn 760	Lys	Pro	Glu	Pro	Gly 7 6 5	Asp	Ala	Leu
	Gln	Gly 770	Leu	Asn	Lys	Asp	Asp 775	Lys	Ala	Gln	Ala	Met 780	Ala	Val	Ile	Gly
55	Val 785	Asn	Lys	Tyr	Leu	Ala 790	Leu	Thr	Glu	Lys	Gly 795	Asp	Ile	Arg	Ser	Phe 800
60	Glm	Ile	Lys	Pro	Gly 805	Thr	Gln	Gln	Leu	Glu 810	Arg	Pro	Ala	Gln	Thr 815	Leu
	Ser	Arg	Glu	Gly 820	Ile	Ser	Gly	Glu	Leu 825	Lys	Asp	Ile	His	Val 830	Asp	His
65	Lys	Gln	Asn 835	Leu	Tyr	Ala	Leu	Thr 840	His	Glu	Gly	Glu	Val 845	Phe	His	Gln

	Pro	Arg 850	Glu	Ala	Trp	Gln	Asn 855	Gly	Ala	Glu	Ser	Ser 860	Ser	Trp	His	Lys
5	Leu 865	Ala	Leu	Pro	Gln	Ser 870	Glu	Ser	Lys	Leu	Lys 875	Ser	Leu	Asp	Met	Ser 880
	His	Glu	His		Pro 885	Ile	Ala	Thr	Phe	Glu 890	Asp	Gly	Ser	Gln	His 895	Gln
10	Leu	Lys	Ala	Gly 900	Gly	Ттр	His	Ala	Tyr 905	Ala	Ala	Pro	Glu	Arg 910	Gly	Pro
	Leu	Ala	Val 915	Gly	Thr	Ser	Gly	Ser 920	Gln	Thr	Val	Phe	Asn 925	Arg	Leu	Met
15	Gln	Gly 930	Val	Lys	Gly	Lys	Val 935	Ile	Pro	Gly	Ser	Gly 940	Leu	Thr	Val	Lys
20	Leu 945	Ser	Ala	Gln	Thr	Gly 950	Gly	Met	Thr	Gly	Ala 955	Glu	Gly	Arg	Lys	Val 960
	Ser	Ser	Lys	Phe	Ser 965	Glu	Arg	Ile	Arg	Ala 970	Tyr	Ala	Phe	Asn	Pro 975	Thr
25	Met	Ser	Thr	Pro 980	Arg	Pro	Ile	Lys	Asn 985	Ala	Ala	Тут	Ala	Thr 990	Gln	His
30	Gly	Trp	Gln 995	Gly	Arg	Glu	Gly	Leu 1000	Lys)	Pro	Leu	Tyr	Glu 1005	Met	Gln	Gly
	Ala	Leu 1010	Ile O	Lys	Gln	Leu	Авр 1019	Ala	His	Asn	Val	Arg 1020	His)	Asn	Ala	Pro
35	Gln 102!		Asp	Leu	Gln	Ser 1030	Lys	Leu	Glu	Thr	Leu 1039	Asp 5	Leu	Gly	Glu	His 1040
	Gly	Ala	Glu	Leu	Leu 1045		Авр	Met	Lys	Arg 1050	Phe)	Arg	Asp	Glu	Leu 1055	Glu i
40			Ala	1060	D				106	5				107	0	
45	Leu	Lув	Ser 107	Asn 5	Gly	Glu	Ile	Asn 108	Ser	Glu	Phe	Lys	Pro 108	Ser 5	Pro	Gly
7.7	Lys	Ala 109		Val	Gln	Ser	Phe 109	Asn 5	Val	Asn	Arg	Ser 110	Gly	Gln	Авр	Leu
50	Ser 110		Ser	Leu	Gln	Gln 111		Val	His	Ala	Thr 111	Pro 5	Pro	Ser	Ala	Glu 1120
	Ser	Lys	Leu	Gln	Ser 112	Met 5	Leu	Gly	His	Phe 113	Val O	Ser	Ala	Gly	Val 113	Asp 5
55	Met	Ser	His	Gln 114		Gly	Glu	Ile	Pro 114	Leu 5	Gly	Arg	Gln	Arg 115	Asp 0	Pro
60	Asn	Asp	Lys 115		Ala	Leu	Thr	Lys 116	Ser 0	Arg	Leu	Ile	Leu 116	Asp 5	Thr	Val
00	Thr	11e 117		Glu	Leu	His	Glu 117	Leu 5	Ala	Авр	Lys	Ala 118	Lys 0	Leu	Val	Ser
65	Asp 118		Lys	Pro	Asp	Ala 119		Gln	Ile	Lys	Gln 119	Leu 5	Arg	Gln	Gln	Phe 1200

	Авр	Thr	Leu	Arg	Glu 1205		Arg	Tyr	Glu	Ser 121	Asn	Pro	Val	Lys	His 1215	Tyr
5	Thr	Asp	Met	Gly 1220		Thr	His	Asn	Lys 1225		Leu	Glu	Ala	Asn 1230		Asp
10	Ala	Val	Lys 1235		Phe	Ile	Asn	Ala 1240		Lys	Lys	Glu	Нів 1249		Gly	Val
10	Asn	Leu 1250		Thr	Arg	Thr	Val 1255		Glu	Ser	Gln	Gly 1260		Ala	Glu	Leu
15	Ala 126	Lys	Lys	Leu	Lys	Asn 1270		Leu	Leu	Ser	Leu 1279	Asp	Ser	Gly	Glu	Ser 1280
	Met	Ser	Phe	Ser	Arg 1285		Tyr	Gly	Gly	Gly 1290		Ser	Thr	Val	Phe 1295	
20	Pro	Thr	Leu	Ser 1300		Lув	Val	Pro	Val 1305		Val	Ile	Pro	Gly 1310	Ala	Gly
25	Ile	Thr	Leu 1315		Arg	Ala	Tyr	Asn 1320		Ser	Phe	Ser	Arg 1325		Ser	Gly
23	Gly	Leu 1330		Val	Ser	Phe	Gly 1335		Asp	Gly	Gly	Val 1340		Gly	Asn	Ile
30	Met 1345	Val	Ala	Thr		His 1350		Val	Met	Pro	Tyr 1359	Met	Thr	Gly	Lys	Lys 1360
		Ser			1365	;				1370)				1375	•
35	Ser	Pro	Asp	Leu 1380	Ar g	Ile	Gly	Ala	Ala 1385	Val	Ser	Gly	Thr	Leu 1390	Gln	Gly
40		Leu	1395	;				1400)				1405	5		
		Phe 1410)				1415	5				1420)			
45	1425					1430	}				1439	5				1440
		Ser			1445	•				1450	3				1455	5
50		Asn		1460)				1465	5				1470)	
55	Ala	Gly	Leu 1475		Ala	Ser	Ala	Asn 1480		Ala	Ala	Gly	Ser 148		Glu	Arg
		Thr 1490)		•		1495	5				1500)			
60	Arg 1505		Thr	Phe	Leu	Asn 1510		Val	Gly	Ala	Gly 1515		Asn	Leu	Thr	Ala 1520
	Ala	Leu	Gly													Gly

	Ile	Phe	Pro	Ala 1540		Thr	Ser	Thr	Asn 1545	Val	Ser	Ala	Ala	Leu 1550	Ala	Leu
5	qaA	asn	Arg 1555		Ser	Gln	Ser	Ile 1560		Leu	Glu	Leu	Lys 1565		Ala	Glu
	Pro	Val 1570	Thr	Ser	Asn	Asp	Ile 1575		Glu	Leu	Thr	Ser 1580	Th r	Leu	Gly	Lys
10	His 1585		Lys	Asp	Ser	Ala 1590		Thr	Lys	Met	Leu 1595	Ala	Ala	Leu	Lys	Glu 1600
	Leu	Asp	Ąsp	Ala	Lys 1609	Pro	Ala	Glu	Gln	Leu 1610	His	Ile	Leu	Gln	Gln 1615	His
15	Phe	Ser	Ala	Lув 1620		Val	Val	Gly	Asp 1625		Arg	Tyr	Glu	Ala 1630	Val	Arg
20	Asn	Leu	Lys 1635		Leu	Val	Ile	Arg 1640		Gln	Ala	Ala	Asp 1645	Ser	His	Ser
	Met	Glu 1650	Leu)	Gly	Ser	Ala	Ser 1655		Ser	Thr	Thr	Tyr 1660		Asn	Leu	Ser
25	Arg 1665		Asn	Asn	Asp	Gly 1670	Ile	Val	Glu	Leu	Leu 1679	His	Lys	aiH	Phe	Asp 1680
30	Ala	Ala	Leu	Pro	Ala 1685		Ser	Ala	Lys	Arg 1690	Leu)	Gly	Glu	Met	Met 1695	Asn
30	Asn	Asp	Pro	Ala 1700		Lys	Asp	Ile	Ile 1705	Lys	Gln	Leu	Gln	Ser 1710	Thr	Pro
35	Phe	Ser	Ser 1715		Ser	Val	Ser	Met 1720	Glu)	Leu	Lys	Asp	Gly 1725	Leu	Arg	Glu
	Gln	Thr 1736	Glu D	Lys	Ala	Ile	Leu 1735	qaA 5	Gly	Lys	Val	Gly 174	Arg	Glu	Glu	Val
40	Gly 174		Leu	Phe	Gln	Asp 1750		Asn	Asn	Leu	Arg 175	Val 5	Lys	Ser	Val	Ser 1760
45	Val	Ser	Gln	ser	Val 176		Lys	Ser	Glu	Gly 1770	Phe	Asn	Thr	Pro	Ala 1779	Leu
40	Leu	Leu	Gly	Thr 178		Asn	Ser	Ala	Ala 178		Ser	Met	Glu	Arg 1790	Asn O	Ile
50	Gly	Thr	Ile 179		Phe	Lys	Tyr	Gly 180	Gln D	Asp	Gln	Asn	Thr 180	Pro 5	Arg	Arg
	Phe	Thr 181	Leu 0	Glu	Gly	Gly	Ile 181		Gln	Ala	Asn	Pro 182	Gln 0	Val	Ala	Ser
55	Ala 182		Thr	Авр	Leu	Lys 183		Glu	Gly	Leu	Glu 183	Met 5	Lys	'Ser		

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

PCT/US99/26039

	ATGACATCGT	CACAGCAGCG	GGTTGAAAGG	TTTTTACAGT	ATTTCTCCGC	CGGGTGTAAA	60
	ACGCCCATAC	ATCTGAAAGA	CGGGGTGTGC	GCCCTGTATA	ACGAACAAGA	TGAGGAGGCG	120
3	GCGGTGCTGG	AAGTACCGCA	ACACAGOGAC	AGCCTGTTAC	TACACTGCCG	AATCATTGAG	180
	GCTGACCCAC	AAACTŢCAAT	AACCCTGTAT	TCGATGCTAT	TACAGCTGAA	TTTTGAAATG	240
10	GCGGCCATGC	GCGGCTGTTG	GCTGGCGCTG	GATGAACTGC	ACAACGTGCG	TTTATGTTTT	300
	CAGCAGTCGC	TGGAGCATCT	GGATGAAGCA	AGTTTTAGCG	ATATCGTTAG	CGGCTTCATC	360
	GAACATGCGG	CAGAAGTGCG	TGAGTATATA	GCGCAATTAG	ACGAGAGTAG	CGCGGCATAA	420
16							

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This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

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20	Met 1	Thr	Ser	Ser	Gln 5	Gln	Arg	Val	Glu	Arg 10	Phe	Leu	Gln	Tyr	Phe 15	Ser
25	Ala	Gly	Сув	Lys 20	Thr	Pro	Ile	His	Leu 25	Lys	Ąsp	Gly	Val	Сув 30	Ala	Leu
	Tyr	Asn	Glu 35	Gln	Asp	Glu	Glu	Ala 40	Ala	Val	Leu	Glu	V al 4 5	Pro	Gln	His
30	Ser	Asp 50	Ser	Leu	Leu	Leu	His 55	Cys	Arg	Ile	Ile	Glu 60	Ala	Asp	Pro	Gln
25	Thr 65	Ser	Ile	Thr	Leu	Тут 70	Ser	Met	Leu	Leu	Gln 75	Leu	Asn	Phe	Glu	Met 80
35	Ala	Ala	Met	Arg	Gly 85	Cys	Trp	Leu	Ala	Leu 90	Asp	Glu	Leu	aiH	Asn 95	Val
40	Arg	Leu	Cys	Phe 100	Gln	Gln	Ser	Leu	Glu 105	His	Leu	Asp	Glu	Ala 110	Ser	Phe
	Ser	Asp	Ile 115	Val	Ser	Gly	Phe	Ile 120	Glu	His	Ala	Ala	Glu 125	Val	Arg	Glu
45	Tyr	Ile	Ala	Gln	Leu	ĄaĄ	Glu 135	Ser	Ser	Ala	Ala					

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

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The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met 55 1 5 10 15

	Ala	Leu	Val	Leu 20	Val	Arg	Pro	Glu	25	GIu	Thr	Thr	GIY	ser 30	THE	ser
5	Ser	Гуs	Ala 35	Leu	Gln	Glu	Val	Val 40	Val	Lys	Leu	Ala	Glu 45	Glu	Leu	Met
	Arg	Asn 50	Gly	Gln	Leu	Asp	Asp 55	Ser	Ser	Pro	Leu	Gly 60	Lys	Leu	Leu	Ala
	Lys 65	Ser	Met	Ala	Ala	Asp 70	Gly	Lys	Ala	Gly	Gly 75	Gly	Ile	Glu	Asp	Val 80
10	Ile	Ala	Ala	Leu	Asp 85	Lys	Leu	Ile	His	Glu 90	Lys	Leu	Gly	Asp	Asn 95	Phe
	Gly	Ala	Ser	Ala 100	Asp	Ser	Ala	Ser	Gly 105	Thr	Gly	Gln	Gln	Asp 110	Leu	Met
15			115					120					125		Leu	
		130					135					140			Pro	
	145		_			150					155				Phe	160
20	•				165				•	170					Asn 175	
				180					185					190	Ile	
25			195					200					205		Ala	
		210					215					220			Ser	
	225					230					235				Asp	240
30	_				245					250					Ile 255	
				260					265					270		
35			275					280					285		Ala	
		290					295					300			Glu	
	Thr 305	Leu	Lys	qaa	AIa	310 G1A		ınr	GTA	ınr	315	val	GTU	ser	Ser	320

PCT/US99/26039

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg 325 330 335

Asn Gln Ala Ala Ala 340

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This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin_{Ps}: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

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ATGCAGAGTC	TCAGTCTTAA	CAGCAGCTCG	CTGCAAACCC	CGGCAATGGC	CCTTGTCCTG	60
GTACGTCCTG	AAGCCGAGAC	GACTGGCAGT	ACGTOGAGCA	AGGCGCTTCA	GGAAGTTGTC	120
GTGAAGCTGG	CCGAGGAACT	GATGCGCAAT	GGTCAACTCG	ACGACAGCTC	GCCATTGGGA	180
AAACTGTTGG	CCAAGTCGAT	GGCCGCAGAT	GGCAAGGCGG	GCGGCGGTAT	TGAGGATGTC	240
ATCGCTGCGC	TGGACAAGCT	GATCCATGAA	AAGCTCGGTG	ACAACTTCGG	CGCGTCTGCG	300
GACAGCGCCT	CGGGTACCGG	ACAGCAGGAC	CTGATGACTC	AGGTGCTCAA	TGGCCTGGCC	360
AAGTCGATGC	TOGATGATCT	TCTGACCAAG	CAGGATGGCG	GGACAAGCTT	CTCCGAAGAC	420
GATATGCCGA	TGCTGAACAA	GATCGCGCAG	TTCATGGATG	ACAATCCCGC	ACAGTTTCCC	480
AAGCCGGACT	CGGGCTCCTG	GGTGAACGAA	CTCAAGGAAG	ACAACTTCCT	TGATGGCGAC	540
GAAACGGCTG	CGTTCCGTTC	GGCACTCGAC	ATCATTGGCC	AGCAACTGGG	TAATCAGCAG	600
agtgacgctg	GCAGTCTGGC	AGGGACGGGT	GGAGGTCTGG	GCACTCCGAG	CAGTTTTTCC	660
AACAACTCGT	CCGTGATGGG	TGATCCGCTG	ATCGACGCCA	ATACCGGTCC	CGGTGACAGC	720
GGCAATACCC	GTGGTGAAGC	GGGGCAACTG	ATCGGCGAGC	TTATCGACCG	TGGCCTGCAA	780
TCGGTATTGG	COGGTGGTGG	ACTGGGCACA	CCCGTAAACA	CCCCGCAGAC	CGGTACGTCG	840
GCGAATGGCG	GACAGTCCGC	TCAGGATCTT	GATCAGTTGC	TGGGCGGCTT	GCTGCTCAAG	900
GGCCTGGAGG	CAACGCTCAA	GGATGCCGGG	CAAACAGGCA	CCGACGTGCA	GTCGAGCGCT	960
GCGCAAATCG	CCACCTTGCT	GGTCAGTACG	CTGCTGCAAG	GCACCCGCAA	TCAGGCTGCA	1020
GCCTGA						1026

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817, which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

	TCCACTTCGC	TGATTTTGAA	ATTGGCAGAT	TCATAGAAAC	GTTCAGGTGT	GGAAATCAGG	60
10	CTGAGTGCGC	AGATTTCGTT	GATAAGGGTG	TGGTACTGGT	CATTGTTGGT	CATTTCAAGG	120
10	CCTCTGAGTG	CGGTGCGGAG	CAATACCAGT	CTTCCTGCTG	GCGTGTGCAC	ACTGAGTCGC	180
	AGGCATAGGC	ATTTCAGTTC	CTTGCGTTGG	TTGGGCATAT	AAAAAAGGA	ACTTTTAAAA	240
15	ACAGTGCAAT	GAGATGCCGG	CAAAACGGGA	ACCGGTCGCT	GCGCTTTGCC	ACTCACTTCG	300
	AGCAAGCTCA	ACCCCAAACA	TCCACATCCC	TATCGAACGG	ACAGCGATAC	GGCCACTTGC	360
20	TCTGGTAAAC	CCTGGAGCTG	GCGTCGGTCC	AATTGCCCAC	TTAGCGAGGT	AACGCAGCAT	420
20	GAGCATCGGC	ATCACACCCC	GGCCGCAACA	GACCACCACG	CCACTCGATT	TTTCGGCGCT	480
	AAGCGGCAAG	AGTCCTCAAC	CAAACACGTT	CGGCGAGCAG	AACACTCAGC	AAGCGATCGA	540
25	CCCGAGTGCA	CTGTTGTTCG	GCAGCGACAC	ACAGAAAGAC	GTCAACTTCG	GCACGCCCGA	600
	CAGCACCGTC	CAGAATCCGC	AGGACGCCAG	CAAGCCCAAC	GACAGCCAGT	CCAACATCGC	660
30	TAAATTGATC	AGTGCATTGA	TCATGTCGTT	GCTGCAGATG	CTCACCAACT	CCAATAAAAA	720
30	GCAGGACACC	AATCAGGAAC	AGCCTGATAG	CCAGGCTCCT	TTCCAGAACA	ACGGCGGGCT	780
	CGGTACACOG	TOGGCOGATA	GCGGGGGCGG	CGGTACACCG	GATGCGACAG	GTGGCGGCGG	840
35	CGGTGATACG	CCAAGOGCAA	CAGGCGGTGG	CGGCGGTGAT	ACTCCGACCG	CAACAGGCGG	900
	TGGCGGCAGC	CGTGGCGGCG	GCACACCCAC	TGCAACAGGT	GGCGGCAGCG	GTGGCACACC	960
40	CACTGCAACA	GCCGGTGGCG	AGGGTGGCGT	AACACCGCAA	ATCACTCCGC	AGTTGGCCAA	1020
40	CCCTAACCGT	ACCTCAGGTA	CTGGCTCGGT	GTCGGACACC	GCAGGTTCTA	CCGAGCAAGC	1080
	CGGCAAGATC	AATGTGGTGA	AAGACACCAT	CAAGGTCGGC	GCTGGCGAAG	TCTTTGACGG	1140
45	CCACGGCGCA	ACCTTCACTG	CCGACAAATC	TATGGGTAAC	GGAGACCAGG	GCGAAAATCA	1200
	GAAGCCCATG	TTCGAGCTGG	CTGAAGGCGC	TACGTTGAAG	AATGTGAACC	TGGGTGAGAA	1260
50	CGAGGTCGAT	GGCATCCACG	TGAAAGCCAA	AAACGCTCAG	GAAGTCACCA	TTGACAACGT	1320
50	GCATGCCCAG	AACGTCGGTG	AAGACCTGAT	TACGGTCAAA	GGCGAGGGAG	GCGCAGCGGT	1380
	CACTAATCTG	AACATCAAGA	ACAGCAGTGC	CAAAGGTGCA	GACGACAAGG	TTGTCCAGCT	1440
55	CAACGCCAAC	ACTCACTTGA	AAATCGACAA	CTTCAAGGCC	GACGATTTCG	GCACGATGGT	1500
	TCGCACCAAC	GGTGGCAAGC	agtttgatga	CATGAGCATC	GAGCTGAACG	GCATCGAAGC	1560
60	TAACCACGGC	AAGTTOGCCC	TGGTGAAAAG	CGACAGTGAC	GATCTGAAGC	TGGCAACGGG	1620

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							•	- 25 -							•	
	CAACATCGCC	ATG	ACCG	ACG I	CAAA	CACGO	CTA	CGATA	AA A	CCCAC	GCAT	CGAC	CCAA	.CA	168	0
	CACCGAGCTT	TGA	ATCC	AGA C	AAGT	AGCTI	GAA	AAAAC	GG G	GTGG/	CTC				172	9
5																
3	This DNA	mole	cule	is kn	own	as the	dsp	E ger	e for	Psei	ıdom	onas	syrin	gae.	This	
	isolated Di	NA π	olec	ule o	fthe	prese	nt in	venti	on en	code	s a pr	otein	or p	olype	ptide	;
	which elic	its a p	olant	patho	gen'	s hyp	erser	ısitiv	e res	ponse	havi	ng ar	ami	no ac	id	
	sequence o	f SE	Q. ID	No.	. 14 a	s foll	ows:									
10	Met 1	Ser	Ile	Gly	Ile 5	Thr	Pro	Arg	Pro	Gln 10	Gln	Thr	Thr	Thr	Pro 15	Leu
15	Asp	Phe	Ser	Ala 20	Leu	Ser	Gly	Lys	Ser 25	Pro	Gln	Pro	Asn	Tar 30	Phe	Gly
	Glu	Gln	Asn 35	Thr	Gln	Gln	Ala	Ile 40	Asp	Pro	Ser	Ala	Leu 45	Leu	Phe	Gly
20	Ser	Asp 50	Thr	Gln	Lys	Asp	Val 55	Asn	Phe	Gly	Thr	Pro 60	Asp	Ser	Thr	Val
25	Gln 65	Asn	Pro	Gln	Asp	Ala 70	Ser	Lys	Pro	Asn	Asp 75	Ser	Gln	ser	Asn	11e 80
25	Ala	Lys	Leu	Ile	Ser 85	Ala	Leu	Ile	Met	Ser 90	Leu	Leu	Gln	Met	Leu 95	Thi
30	Asn	Ser	Asn	Lys 100	Lys	Gln	Asp	Thr	Asn 105	Gln	Glu	Gln	Pro	Asp 110	Ser	Glı
	Ala	Pro	Phe 115	Gln	Asn	Asn	Gly	Gly 120	Leu	Gly	Thr	Pro	Ser 125	Ala	Asp	Sei
35	Gly	Gly 130	Gly	Gly	Thr	Pro	Asp 135	Ala	Thr	Gly	Gly	Gly 140	Gly	Gly	Asp	Th
							-3	63	 1		777b	Dec	The	nl a	Thr	G)

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly 145 150 150 160 Gly Gly Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly 170 Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Gly Gly Val Thr

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr 200

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile 50

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp 225 230 235 240

	Gly	His	Gly	Ala	Thr 245	Phe	Thr	Ala	Двр	Lys 250	Ser	Met	Gly	Asn	Gly 255	Asp
5	Gln	Gly	Glu	Asn 260	Gln	Lys	Pro	Met	Phe 265	Glu	Leu	Ala	Glu	Gly 270	Ala	Thr
	Leu	Lys	Asn 275	Val	Asn	Leu	Gly	Glu 280	Asn	Glu	Val	Asp	Gly 285	Ile	His	Val
10	Lys	Ala 290	Lys	Asn	Ala	Gln	Glu 295	Val	Thr	Ile	Asp	Asn 300	Val	His	Ala	Gln
15	Asn 305	Val	Gly	Glu	Asp	Leu 310	Ile	Thr	Val	Lys	Gly 315	Glu	Gly	Gly	Ala	Ala 320
15	Val	Thr	Àsn	Leu	Asn 325	Ile	Lys	Asn	Ser	Ser 330	Ala	Lys	Gly	Ala	Asp 335	Asp
20	Lys	Val	Val	Gln 340	Leu	Asn	Ala	Asn	Thr 345	His	Leu	Lys	Ile	Asp 350	Asn	Phe
	Lys	Ala	Asp 355	Asp	Phe	Gly	Thr	Met 360	Val	Arg	Thr	Asn	Gly 365	Gly	Lys	Gln
25	Phe	Asp 370	Asp	Met	Ser	Ile	Glu 375	Leu	Asn	Gly	Ile	Glu 380	Ala	Asn	His	Gly
30	Lys 385	Phe	Ala	Leu	Val	Lys 390	Ser	Asp	Ser	Asp	Asp 395	Leu	Lys	Leu	Ala	Thr 400
<i></i>	Gly	Asn	Ile	Ala	Met 405	Thr	Asp	Val	Lys	His 410	Ala	Tyr	Asp	Lys	Thr 415	Gln
35	Ala	Ser	Thr	Gln 420	His	Thr	Glu	Leu								

This protein or polypeptide is about 42.9 kDa.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

45 Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1 Ser Val Gly Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
35 Ash Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly

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	Asn 65	Thr	Gly	Asn	Ala	Pro 70	Ala	Lys	Asp	Gly	Asn 75	Ala	Asn	Ala	Gly	Ala 80
•	Asn	Asp	Pro	Ser	Lys 85	aeA	Asp	Pro	Ser	Lys 90	Ser	Gln	Ala	Pro	Gln 95	Ser
5	Ala	Asn	Lys	Thr 100	Gly	Asn	Val	Ąsp	Asp 105	Ala	Asn	Asn	Gln	Asp 110	Pro	Met
	Gln	Ala	Leu 115	Met	Gln	Leu	Leu	Glu 120	Asp	Leu	Val	Lys	Leu 125	Leu	Lys	Ala
10	Ala	Leu 130	His	Met	Gln	Gln	Pro 135	Gly	Gly	Asn	Asp	Lys 140	Gly	Asn	Gly	Val
	Gly 145	Gly	Ala	Asn	Gly	Ala 150	Lys	Gly	Ala	Gly	Gly 155	Gln	Gly	Gly	Leu	Ala 160
					165					170					Gly 175	
15	_			180					185					190	Gly	
			195					200					205		Gly	
20	_	210					215					220			Gln	
	Ala 225	Gly	Asp	Val	Asn	Gly 230	Ala	Asn	Gly	Ala	Asp 235	Asp	Gly	Ser	Glu	Asp 240
	Gln	Gly	Gly	Leu	Thr 245	Gly	Val	Leu	Gln	Lys 250	Leu	Met	Lys	Ile	Leu 255	Asn
25	Ala	Leu	Val	Gln 260	Met	Met	Gln	Gln	Gly 265	Gly	Leu	Gly	Gly	Gly 270	Asn	Gln
	Ala	Gln	Gly 275	Gly	Ser	Lys	Gly	Ala 280	Gly	Asn	Ala	Ser	Pro 285	Ala	Ser	Gly
30	Ala	Asn 290		Gly	Ala	Asn	Gln 295	Pro	Gly	Ser	Ala	Asp 300	Asp	Gln	Ser	Ser
	Gly 305		Asn	Asn	Leu	Gln 310	Ser	Gln	Ile	Met	Asp 315	Val	Val	Lys	Glu	Val 320
	Val	Gln	Ile	Leu	Gln 325	Gln	Met	Leu	Ala	Ala 330	Gln	Asn	Gly	Gly	Ser 335	Gln
35	Gln	Ser	Thr	Ser 340	Thr	Gln	Pro	Met								

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 16 as follows:

	ATGTCAGTCG	GAAACATCCA	GAGCCCGTCG	AACCTCCCGG	GTCTGCAGAA	CCTGAACCTC	60
	AACACCAACA	CCAACAGCCA	GCAATCGGGC	CAGTCCGTGC	AAGACCTGAT	CAAGCAGGTC	120
	GAGAAGGACA	TCCTCAACAT	CATCGCAGCC	CTCGTGCAGA	AGGCCGCACA	GTCGGCGGGC	180
	GGCAACACCG	GTAACACCGG	CAACGCGCCG	GCGAAGGACG	GCAATGCCAA	CGCGGGCGCC	240
5	AACGACCCGA	GCAAGAACGA	CCCGAGCAAG	AGCCAGGCTC	CGCAGTCGGC	CAACAAGACC	300
	GGCAACGTCG	ACGACGCCAA	CAACCAGGAT	CCGATGCAAG	CGCTGATGCA	GCTGCTGGAA	360
	GACCTGGTGA	AGCTGCTGAA	GGCGGCCCTG	CACATGCAGC	AGCCCGGCGG	CAATGACAAG	420
	GGCAACGGCG	TGGGCGGTGC	CAACGGCGCC	AAGGGTGCCG	GCGGCCAGGG	CGGCCTGGCC	480
	GAAGCGCTGC	AGGAGATCGA	GCAGATCCTC	GCCCAGCTCG	cccccccc	TGCTGGCGCC	540
10	GGCGGCGCGG	GTGGCGGTGT	CCCCCCTCCT	GCTGGCGGG	ATGGCGGCTC	CGGTGCGGGT	600
	GGCGCAGGCG	GTGCGAACGG	CGCCGACGGC	GGCAATGGCG	TGAACGGCAA	CCAGGCGAAC	660
	GGCCCGCAGA	ACGCAGGCGA	TGTCAACGGT	GCCAACGGCG	CGGATGACGG	CAGCGAAGAC	720
	CAGGGCGGCC	TCACCGGCGT	GCTGCAAAAG	CTGATGAAGA	TCCTGAACGC	GCTGGTGCAG	780
	ATGATGCAGC	AAGGCGGCCT	CGGCGGCGGC	AACCAGGCGC	AGGGCGGCTC	GAAGGGTGCC	B40
15	GGCAACGCCT	OGCOGGCTTC	CGGCGCGAAC	CCGGGGGGCGA	ACCAGCCCGG	TTCGGCGGAT	900
	GATCAATCGT	CCGGCCAGAA	CAATCTGCAA	TCCCAGATCA	TGGATGTGGT	GAAGGAGGTC	960
	GTCCAGATCC	TGCAGCAGAT	GCTGGCGGCG	CAGAACGGCG	GCAGCCAGCA	GTCCACCTCG	1020
	ACGCAGCCGA	TGTAA					1035

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Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from Xanthomonas campestris pv. glycines has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

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Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala 1 5 10 15

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Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr 20 25

This sequence is an amino terminal sequence having only 26 residues from the hypersensitive response elicitor polypeptide or protein of Xanthomonas campestris pv. glycines. It matches with fimbrial subunit proteins determined in other Xanthomonas campestris pathovars.

The hypersensitive response elicitor polypeptide or protein from

Xanthomonas campestris pv. pelargonii is heat stable, protease sensitive, and has a
molecular weight of 20 kDa. It includes an amino acid sequence corresponding to

SEQ. ID. No. 18 as follows:

Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln

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Leu Leu Ala Met
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Isolation of Erwinia carotovora hypersensitive response elictor protein or polypeptide is described in Cui et al., "The RsmA Mutants of Erwinia carotovora subsp. carotovora Strain Ecc71 Overexpress hrp N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of Erwinia stewartii is set forth in Ahmad et al., "Harpin is Not Necessary for the Pathogenicity of Erwinia stewartii on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of Erwinia stewartii on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma, and Phytophora citrophthora are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and

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Acquired Resistance in Tobacco," <u>Eur. J. Biochem.</u>, 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," <u>Plant Path.</u> 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," <u>Plant J.</u>, 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," <u>Eur. J. Plant Path.</u>, 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. sepedonicus which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed in vitro or in vivo in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

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In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do not elicit a hypersensitive response include fragments of the *Erwinia*. Suitable fragments include a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 169 and 403, 210 and 403, 267 and 403, or 343 and 403. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 105 and 179, 137 and 166, 121 and 150, or 137 and 156. Other suitable fragments can be identified in accordance with the present invention.

Another example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the

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following amino acids of SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

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Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 µm g/ml E. coli DNA.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

The hypersensitive response elicitor of the present invention is preferably in isolated form (i.e. separated from its host organism) and more preferably produced in purified form (preferably at least about 60%, more preferably 80%, pure) by conventional techniques. Typically, the hypersensitive response elicitor of the present invention is produced but not secreted into the growth medium of recombinant host cells. Alternatively, the protein or polypeptide of the present invention is secreted into growth medium. In the case of unsecreted protein, to isolate the protein, the host cell (e.g., *E. coli*) carrying a recombinant plasmid is propagated, lysed by sonication, heat, or chemical treatment, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to heat treatment and the hypersensitive response elicitor is separated by centrifugation. The supernatant fraction containing the hypersensitive response elicitor is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the fragment. If necessary, the protein fraction may be further purified by ion exchange or HPLC.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant DNA technology. Generally, this involves inserting the DNA molecule into an

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expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the proteinencoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA;

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microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promotors such as the T7 phage promotor, *lac* promotor,

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trp promotor, recA promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promotor or other E. coli promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

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The present invention's method of imparting stress resistance to plants can involve applying the hypersensitive response elicitor polypeptide or protein in a non-infectious form to all or part of a plant or a plant seed under conditions effective for the elicitor to impart stress resistance. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart stress resistance in plants.

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As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart stress resistance in plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart stress resistance to plants. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart stress resistance to plants.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant or plant seed can be carried out in a number of ways, including: 1) application of an isolated hypersensitive response elicitor or 2) application of bacteria which do not cause disease and are transformed with a genes encoding the elicitor. In the latter embodiment, the elicitor can be applied to plants or plant seeds by applying bacteria containing the DNA molecule encoding a hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the elicitor so that the elicitor can contact plant or plant seed cells. In these embodiments, the elicitor is produced by the bacteria *in planta* or on seeds or just prior to introduction of the bacteria to the plants or plant seeds.

The methods of the present invention can be utilized to treat a wide variety of plants or their seeds to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea,

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chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

In accordance with the present invention, the term "stress" refers to drought, salt, cold temperatures (e.g., frost), chemical treatment (e.g., insecticides, fungicides, herbicides, fertilizers), water, excessive light, and insufficient light.

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The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds or propagules (e.g., cuttings), in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide, in accordance with present invention, can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the elicitor with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart stress resistance to plants.

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The hypersensitive response elicitor polypeptide or protein, in accordance with the present invention, can be applied to plants or plant seeds alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor

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polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM of the elicitor.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof. Suitable fertilizers include (NH₄)₂NO₃. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding such an elicitor are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA.

Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby

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incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies.

Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with Agrobacterium tumefaciens or A. rhizogenes previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the Gram-negative family Rhizobiaceae. Its species are responsible for crown gall (A. tumefaciens) and hairy root disease (A. rhizogenes). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a

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convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of A. tumefaciens or the Ri plasmid of A. rhizogenes. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures. Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the

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gene encoding the hypersensitive response elicitor resulting in stress resistance to the plant. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart stress resistance to plants. While not wishing to be bound by theory, such stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor in accordance with the present invention is applied. These other materials, including a hypersensitive response elicitor in accordance with the present invention, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor in accordance with the present invention to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Hypersensitive Response Elicitor-Treated Cotton is More Resistant to the Damage Caused by Insecticide Stress

Aphids (Aphids gossypii) infect cotton during the entire growth season. The damage of aphid infection ranges from honeydew deposit that contaminates the lint and reduces crop value to defoliation that reduces or destroys crops. To protect plants from aphid infection, cotton is usually sprayed with insecticides, for example Asana XL when the infection pressure is not very high, and Admire when the infestation pressure is high. The effect of a hypersensitive response elicitor on aphids in cotton was studied by a trial involving a randomized complete block design. This

involved treatment with *Erwinia amylovora* hypersensitive response elicitor (i.e. HP-1000TM) at 20, 60, and 80 ppm and a chemical insecticide, Asana XL, at 8 oz./ac. Each treatment involved foliar application beginning at cotyledon to three true leaves and thereafter at 14 day intervals using a backpack sprayer. Aphid counts and overall growth of the cotton were made immediately prior to spray application at 14, 28, 35, and 42 days after the first treatment ("DAT 1"). Twenty-five randomly selected leaves per plot were collected at the first three sampling dates and the leaves per plot at the final sampling date.

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1. Aphid control: The number of aphids in the hypersensitive response elicitor-treated cotton were significantly reduced in comparison to the chemical treated cotton (see Table 1).

Table 1. Aphid count per leaf on cotton after treatment with Asana XL[®] or HP-1000™

Number of aphids per leaf

No. sprays applied/days after treatment

eatment Rate² 1/14DAT1 2/28DAT1 3/35DAT1 4/42DAT1

2/28DAT1 3/35DAT1 4/42DAT1

Treatment	Rate ²	1/14DAT1	2/28DAT1	3/35DAT1	4/42DAT1
Asana XL	8 oz/ac	0.2 a	32.2 a	110.0 a	546.9 a
HP-1000™	20 μg/ml	0.2 a	7.8 Ъ	22.9 b	322.1 a
HP-1000™	60 µg/ml	0.1 a	4.9 b	34.6 b	168.3 a
HP-1000™	80 μg/ml	0.0 a	2.7 Ь	25.8 b	510.2 a

¹Means followed by different letters are significantly different according to Duncan's MRT, P=0.05. ²Rate for Asana XL[®] is for formulated product, rate for HP-1000TM is for active ingredient (a.i.).

At 14 days after DAT 1, aphid counts were relatively low across all of the treatments, but by 28 days after DAT 1 (by which time two sprayings had been applied), the number of aphids per leaf were significantly greater in Asana XL-treated plants compared to the hypersensitive response elicitor-treated cottons. By 35 days after DAT 1 (by which time three sprayings had been applied), aphid counts had risen for all treatments, yet aphid counts per leaf were still significantly lower for hypersensitive response elicitor-treated cotton compared to the Asana XL treatment. Finally, at 42 days after DAT 1 (by which time four sprayings had been applied), the number of aphids per leaf had increased to a level that threatened to overwhelm the

plants even when treated with the standard chemical insecticide. To save the trial, an ther chemical, Pravado (Admire), was applied to all plots to eradicate aphids from the field.

resistant to the damage caused by Pravado (Admire) and Asana. After the second chemical spraying, it was observed that cotton plants were stress shocked by the insecticides. The cotton plants previously treated with Asana and untreated control were defoliated. On most of the chemical-treated cotton, there were no leaves, or very few leaves, in the lower portion of plants. However, the hypersensitive response elicitor-treated plants, especially the plot where hypersensitive response elicitor was applied at 80 ppm, had no defoliation and the cotton plants were vigorous and healthy. By counting the number of mature balls, it clearly showed that hypersensitive response elicitor-treated plants (at 80 ppm) had more ball setting than chemical and untreated control (Table 2), indicating that hypersensitive response elicitor-treated plants were more tolerant to the stress caused by insecticide.

Table 2. Number of Formed Cotton Balls Counted on Ten Plants in Each of Four Replicates Per Treatment.

20	Treatment	No. balls/10 plants/replicate
	UTC	28
	Chemical standard	6
	Hypersensitive Response Elicitor	35
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<u>Example 2</u> - Hypersensitive Response Elicitor-Treated Cucumbers are More Resistant to Drought

A cucumber field trial was set up to test the effect of *Erwinia* amylovora hypersensitive response elicitor on disease control, tolerance to drought stress, and yield. Three different rates were tested, there at 15, 30, and 60 µg/ml. In addition to hypersensitive response elicitor treatment, there was an untreated control. Each treatment contained three replicate plots. When the first true leaf emerges, hypersensitive response elicitor was sprayed with a back bag sprayer. The second spray was applied ten days after the first spray. The third application was right after

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the recovery of cucumber seedlings after the transplanting to the field. Individual treatment was randomly assigned in the field.

When the first true leaf emerged (Day 0), a first application was sprayed. Usually cucumber seedlings are transplanted when seedlings show two true leaves. It has been known that the recovery rate after the transplanting is closely related to the size of the seedlings. Because of the drought, the seedlings were maintained in the nursery for an extra ten days and the second spray was applied on Day 10. Two days after the second spray, the plants were transplanted into fields and covered with plastic sheets. The plants had 4 – 5 true leaves.

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Result

The recovery rate of the transplanted cucumber seedlings was higher for the hypersensitive response elicitor-treated plants than for the untreated control. More than 80% of the hypersensitive response elicitor-treated cucumber seedlings survived, while only 57% untreated plants survived.

Throughout the growth season, there was a serious drought problem. Early field visits indicated that hypersensitive response elicitor-treated plants had more root mass and better over-all growth. Hypersensitive response elicitor-treated cucumber started to flower 14 days earlier than untreated control cucumber. The early flowering resulted in an earlier harvest. In the first harvest, more than 0.4 kilograms of cucumber fruits per plant were harvested from the hypersensitive response elicitor-treated cucumbers; however, virtually no fruit was harvested from untreated control. By the end of the season, untreated plants died due to severe drought, but hypersensitive response elicitor-treated plants were still alive and had one more harvest.

The final yield was significantly different between hypersensitive response elicitor-treated and untreated plants. Hypersensitive response elicitor administered at the rate of 30 ppm produced three times greater yield than the control plants (Table 3).

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Table 3. Yield Increase of Cucumber Fruit from Hypersensitive Response Elicitor Treated Plants

Treatment	Replicate	kg/plant	Yield/I	Replicate	% of the Yield Increase
	1	1.25	37.5		
HP 15	II.	1.00	30.0	103.8	241
	III	1.21	36.3		
]	1.54	46.2		
HP 30	П	1.43	42.9	133.2	339
	III	1.47	44.1		
	I	0.43	12.9		
Control	11	0.41	12.3	39.3	
	111	0.47	14.1		

The increased yield was partially attributed to hypersensitive response elicitor-induced growth enhancement and partially resulted from more tolerance of hypersensitive response elicitor-treated cucumber to drought, because usually the yield increase from hypersensitive response elicitor-induced growth enhancement is between 10-40%.

<u>Example 3</u> - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress

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Pepper seedlings were drenched with hypersensitive response elicitor at 20 ppm seven days before transplanting, sprayed seven days after the transplanting, and then, sprayed every fourteen days. Standard chemicals, Brave, Maneb, Kocide, and Admire, were used for the rest of the treatment. In addition to early growth enhancement, which resulted in a higher yield, larger fruit, and resistance to several diseases, hypersensitive response elicitor-treated pepper was more tolerant to herbicide damage. The pepper field was applied with the herbicide SENCOR which is not labeled for pepper. This herbicide is known to cause severe foliar damage to pepper in chemically-treated plants but not with hypersensitive response elicitor-treated plants.

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The difference between the adverse effect of the herbicide on the hypersensitive response elicitor and non-hypersensitive response elicitor treated plants is dramatic. See Table 4 below. Thirty-nine of the 60 elicitor-treated plants showed only minor damage by the herbicide, the damaged leaves were less than 20%. In

contrast, 53 out f the 60 chemically-treated pepper plants had severe damage, 40-57% of the leaves were damaged, and 20 plants were dead. The ability of hypersensitive response elicitors to help crops withstand the phytotoxic effects of a herbicide is very important benefit to in agricultural industry.

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Table 4. Hypersensitive Response Elicitor-Treated Peppers are More Tolerant to Herbicide Damage.

	Treatment	Damage Rating						Damage Index %		
10		1	2	3	4	5	6	41		
	Hypersensitive Response Elicitor	1	38	17	3	1	0			
15	Chemicals 0	1	6	16	19	18		87		
	Damage Rating: 1. No 40-50% leaves damage							20-40% leaves damaged; 4. entire plant dead.		
20	Damage index = sum of each rating times the number of plants under the rating scale, divide by total number of plants times 6.									
	Damage index for hype					treate	d plant	s =		

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<u>Example 4</u> - Hypersensitive Response Elicitor-Treated Pepper is More Tolerant to Herbicide Stress under Controlled Experimental Conditions

A field trial was conducted to test if hypersensitive elicitor treated pepper would be more tolerant to herbicide stress. The trial contains 6 treatments and 4 replicates for each treatment. The treatments are described as follows:

- Control, the peppers were neither treated by a hypersensitive
 response ("HR") elicitor nor by LEXONETM herbicide (DuPont Agricultural Products, Wilmington, Delaware).
 - Control pepper with application of 0.15 pound LEXONE™
 herbicide /acre.
- 3. Control pepper with application of 0.3 pound LEXONE™
 40 herbicide /acre.

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- 4. HR elicitor treatment with no application of LEXONE™ herbicide using a formulated product known as MESSENGER™ biopesticide (Eden Bioscience Corporation, Bothell, Washington) containing 3% HR elicitor protein was used.
- HR elicitor treatment with application of 0.15 pound
 LEXONETM herbicide /acre.
 - 6. HR elicitor treatment with application of 0.3 pound LEXONETM herbicide /acre.

LEXONE™ contains the same active ingredient as SENCOR™ herbicide (Bayer, Kansas City, Missouri) used in Example 3. Pepper seedlings were drenched with MESSENGER™ solution at the concentration of HR elicitor protein of about 20 ppm seven days before transplanting into the field and then sprayed every 14 days after the transplanting. LEXONE was applied at high (0.3 pound/acre) and low levels (0.15 pound/acre). 50 gallon water and 100 mL of the herbicide solution was introduced into the root zone of each plant in the respective treatment five weeks after transplant into the field.

The treatments were evaluated for the percent of chlorosis caused by the LEXONETM herbicide application and for the pepper yield. HR elicitor-treated plants exposed to the high rate of herbicide had significantly less chlorosis and produced 108 % more fruit in comparison to the non-hypersensitive response elicitor treated plants exposed to the same amount of herbicide. See Tables 5 and 6 below. There was no significant difference in the reduction of chlorosis at the low rate of herbicide between the HR elicitor treated and non-HR elicitor treated peppers. However, the HR elicitor treated plants produced 15% more fruit than the corresponding control plants exposed to the same amount of herbicide. There was no chlorosis in either the check or HR elicitor-treated plants that did not receive LEXONETM herbicide treatment.

The HR elicitor treated plants were much less severely affected by the herbicide application than the respective control plants at the high rate of herbicide. However, the amount of visual chlorosis was similar at the low rate for both the check and HR elicitor-treated plants. More importantly, the yields from both the high and low rate herbicide treatments of HR elicitor treated plants were less severely effected

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by the herbicide than the checks. These findings further confirm that HR elicitors can help crops withstand the phytot xic effects of herbicides and are very beneficial to the agricultural industry.

5 Table 5. Reduction of Foliar Chlorosis and Increase in Yield in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONETM Herbicide

ante i e constituiri il la constituiri	NEW COL	Percent f	oliar chlor	osis and yi	eld of pepp	er 🐃	शिक्षावयः १५ ।
Treatment	A	В	C	D	E	Yield (peund)	% difference from the respective control
6 (MESSENGERTM+ High rate LEXONETM)	.13.75	30.00	37.50	36.25	40.00	8.31	108 %
3 (High rate LEXONETA)	26.25	43.75	51.25	50.00	51.25	4.00	- -
5 (MESSENGER TM + low rate LENOXE TM)	16.25	22.50	28.75	23.75	27.50	8.00	15 %
2 (LENOXETM)	12.50	20.00	25.00	25.00	23.75	6.81	-

Table 6. Weight of Harvested Peppers Increased in Hypersensitive Response Elicitor Treated Plants after Exposure to LEXONE™ Herbicide Compared to Check Plants.

Control of the State of the Control of the State of the S	Weight of peppers harvested 12/1/98 in pounds
Treatmest	
HP20 + high rate LEXONE™	8.31
Check + high rate LEXONE	4.00
HP20 + low rate LEXONE™	8.00
Check + low rate LEXONETM	6.81

15 Example 5 - Hypersensitive Response Elicitor-Treated Cotton is More Tolerant to Drought Stress

A non-irrigated cotton trial experienced 26 consecutive days of drought. The average daily heat index was near or over 100 degrees F, adding to the stress placed on the plants in the field.

Observations in the field indicated that plants treated with HR elicitor at the concentration of 15 ppm (2.2 oz formulated product, MESSENGERTM containing 3 % active ingredient HR elicitor protein) were more vigorous and had less defoliation than the check plants as a result of the heat and drought stress. Equal numbers of plants from the MESSENGERTM-treated and the non-MESSENGERTM treated plots were carefully removed from the field and mapped for the number of nodes and bolls by position. The plants were also weighed on a Metler analytical scale to determine whole plant, root and shoot weights.

MESSENGERTM treated plants survived the heat and drought stresses much better than the untreated plants did. Plants treated with MESSENGERTM had 37.6% more root and shoot mass than the check plants (Table 7). The MESSENGERTM treated plants also had significantly more cotton bolls than the check plants (Table 8). The number of cotton bolls from positions 1 and 2 have a significant contribution to the overall yield. Table 8 showed that MESSENGERTM treated plants had 47% more bolls in positions 1 and 2 and 57% more boll from a whole plant in comparison to the yield achieved using a grower standard treatment (i.e. with no MESSENGERTM treatment). A common reaction to stress in cotton is for the plant to abort bolls. The results indicate that MESSENGERTM-treated plants are more tolerant to the drought stress.

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Table 7. Weight per Plant of Non-Irrigated Cotton Following 26 Days of Drought.

Treatment	Root weight (pond/plant)	%Difference	Shoot weight (pond/plant)	% difference	Whole plant weight (pond/plant)	% difference
MESSENGERTM 2.2 oz/acre	0.041 a*	37.6 %	0.505 a	37.5 %	0.546	37.5 %
Control (Grower standard)	0.0298 b		0.367 ь		0.397	
Level of statistically significant	P=0.119	-	P=0.034		·	P=0.033
		1				

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Same letter indicates no statistical difference between the two treatments at the defined level;
 different letter indicates a statistical difference between the two treatments at the defined level.

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Table 8. Number of Bolls per 5 Plants at the Number 1 & 2 positi ns, and Total Number of Bolls from Whole Plants in Non-irrigated Cotton Following 26 days of drought.

Treatment	Avg. # bolls in the #1 & 2 position	Percent difference	Avg. # of total bolls per 5 plant	Percent difference
MESSENGER™2.2	18.4 a	+46.0%	21.4 a	+57.0%
Check	12.6 b		13.6 b	•
Statistically significant level	P=0.032		P=0.01	

Same letter indicates no statistical difference between the two treatments at the defined level;
 different letter indicates a statistical difference between the two treatments at the defined level.

10 <u>Example 6</u> - Hypersensitive Response Elicitor-Treated Tomato is More Tolerant to Calcium Deficiency

Calcium is an important element for plant physiology and development. A deficiency in calcium can cause several plant diseases. For example, blossom-end rot is caused by a localized calcium deficiency in the distal end of the tomato fruit. Because calcium is not a highly mobile element, a deficiency can occur with a fluctuation in water supply. In the past, tomato growers experienced higher level of blossom-end rot during dry weather conditions when infrequent rains storms dumped a lot of water and then return to a hot and dry condition quickly. Lowering or raising the irrigation water table erratically during a dry and hot growing season can also increase the disease.

A field trial was designed to test if HR elicitor protein-treated tornato can be more tolerant to the calcium deficiency under a dry hot growing season.

MESSENGER™, the formulated product containing 3% HR elicitor, was used for the trial. The application rate of the MESSENGER™ was 2.27 oz per care. The first spray of MESSENGER™ was carried out 7 days before the transplanting and then every 14-days after transplanting. MESSENGER™-treated tomatoes were compared with a standard grower treatment not utilizing MESSENGER™. Each treatment had 4 replicates.

The number of infected fruit was counted from a 100 square foot field. The rot typically begins with light tan water soaked lesions, which then enlarge, and then turn black. In a survey, about 20% of the fruits were infected. Severe end-rot

symptoms occurred in the standard treatment; however, an average of only 2.5 % of the fruit was infected in the MESSENGERTM-treated plants. The harvest data showed that MESSENGERTM-treated plants had 8% more marketable fruit (Table 9). The test results demonstrated that MESSENGERTM-treatment can reduce the stress resulting from calcium deficiency and increase plant resistance to blossom-end rot.

Table 9. Hypersensitive Response Elicitor Treatment Reduced Blossom-End Rot Infection, Increased Yield of Tomato Fruit

Treatment	Blosso	m-End Info	ected Fruit*	Tomato Fruit Yield			
	Rep I	Rep II	Rep III	Rep IV	Bin/Acre	% Difference	
MESSENGERTM	0	9	0	1	35	8	
Standard Treatment)	24	22	16	17	31.5	 	

^{*}The data were collected from the fruits in 100 square foot plot

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.

WHAT IS CLAIMED:

- 1. A method of imparting stress resistance to plants comprising:
 applying a hypersensitive response elicitor protein or
 polypeptide in a non-infectious form to a plant or plant seed under conditions
 effective to impart stress resistance.
- A method according to claim 1, wherein the stress resistance is resistance to a stress selected from the group consisting of climated related stress, air pollution stress, chemical stress, and nutritional stress.
 - 3. A method according to claim 2, wherein the stress is chemical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.
- 4. A method according to claim 2, wherein the stress is climaterelated stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.
- 20 5. A method according to claim 2, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.
- 25 6. A method according to claim 2, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.
- 7. A method according to claim 1, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia, Pseudomonas, Xanthamonas, Phythophthera, or Clavibacter.

- 8. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia amylovora, Erwinia carotovora, Erwinia chrysanthemi, and Erwinia stewartii.
- 5 9. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.
- 10. A method according to claim 7, wherein the hypersensitive
 10 response elicitor protein or polypeptide is derived from a Xanthamonas species.
 - 11. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from a Phythophthera.
- 15 12. A method according to claim 7, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Clavibacter michiganesis* subsp. *sepedonicus*.
- 13. A method according to claim 1, wherein plants are treated20 during said applying.
 - 14. A method according to claim 1, wherein plant seeds are treated during said applying, said method further comprising:
 - planting the seeds treated with the hypersensitive response elicitor protein or polypeptide in natural or artificial soil and propagating plants from seeds planted in soil.
- 15. A method according to claim 1, wherein the plant is selected from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,

 peanut, corn, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear,

melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

- 16. A method according to claim 1, wherein the plant is selected
 5 from the group consisting of Arabidopsis thaliana, Saintpaulia, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.
- 17. A method of imparting stress resistance to plants comprising:

 providing a transgenic plant or plant seed transformed with a

 10 DNA molecule which encodes for a hypersensitive response elicitor protein or
 polypeptide and

 growing the transgenic plant or plants produced from the
 transgenic plant seeds under conditions effective to impart stress resistance.
- 15 A method according to claim 17, wherein a transgenic plant is provided.
- 19. A method according to claim 17, wherein a transgenic plant seed is provided, said method further comprising:

 20 planting the transgenic seeds in natural or artificial soil and propagating plants from seeds planted in soil..
- A method according to claim 17, wherein the stress resistance
 is resistance to a stress selected from the group consisting of climated related stress,
 air pollution stress, chemical stress, and nutritional stress.
 - 21. A method according to claim 20, wherein the stress is chemical stress where the chemical is selected from the group consisting of insecticides, fungicides, herbicides, and heavy metals.

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- 22. A method according to claim 20, wherein the stress is climaterelated stress selected from the group consisting of drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light.
- 5 23. A method according to claim 20, wherein the stress is air pollution stress selected from the group consisting of carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, and acidic rain.
- 10 24. A method according to claim 20, wherein the stress is nutritional stress where the nutritional stress is caused by fertilizer, micronutrients, or macronutrients.
- 25. A method according to claim 20, wherein the hypersensitive
 response elicitor protein or polypeptide is derived from Erwinia, Pseudomonas,
 Xanthamonas, Phythophthera, or Clavibacter.
 - 26. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from Erwinia amylovora, Erwinia carotovora, Erwinia chrysanthemi, and Erwinia stewartii.
 - 27. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from *Pseudomonas syringae* or *Pseudomonas solancearum*.
 - 28. A method according to claim 25, wherein the hypersensitive response elicitor protein or polypeptide is derived from a Xanthamonas species.
- 29. A method according to claim 20, wherein the plant is selected
 30 from the group consisting of alfalfa, rice, wheat, barley, rye, cotton, sunflower,
 peanut, com, potato, sweet potato, bean pea, chicory, lettuce, endive, cabbage, brussel
 sprout, beet, parsnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic,

eggplant, pepper, celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane.

5 30. A method according to claim 20, wherein the plant is selected from the group consisting of *Arabidopsis thaliuna*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

PCT/US99/26039 WO 00/28055

SEQUENCE LISTING

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- Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly 65 70 75 80
- Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
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- Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gln Ile Gly Asp 115 120 125
- Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp 130 135 140
- Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala 145 150 155 160
- Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser 165 170 175
- Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro 180 185 190
- Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro 195 200 205
- Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro 210 215 220
- Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly 225 230 235 240
- Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
- Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln 260 265 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn 275 280 285

- Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val 290 295 300
- Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala 305 310 315 320
- Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr 325 330 335
- Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn 340 345 350
- Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp 355 360 365
- Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe 370 375 380
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- His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser 405 410 415
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- Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln 225 230 235 240
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<213> Erwinia amylovora

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<210> 10

<211> 139

<212> PRT

<213> Brwinia amylovora

<400> 10

Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser 10

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu 20

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His 40

Ser Asp Ser Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met 70

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val 90

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe 110 105 100

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu 120

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Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala 135 130

<210> 11

<211> 341

<212> PRT

<213> Pseudomonas syringae

Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser 25

Ser Lys Ala Leu Gln Glu Val Val Lys Leu Ala Glu Glu Leu Met

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala 55

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe 85

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met 105

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met 135

Leu Asn Lys Ile Ala Gln Phe Met Asp Asn Pro Ala Gln Phe Pro 155

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe 165

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile 185

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly

205

195 200

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser 210 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp 245 250 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
275 280 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala 290 295 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala 305 310 315 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg 325 330 335

Asn Gln Ala Ala Ala 340

<210> 12

<211> 1026

<212> DNA

<213> Pseudomonas syringae

<400> 12

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gggcatggagg gacagtccge tcaggatctt gatcagttge tgggcggct gctgctcaag 900
ggcctggagg caacgctcaa ggatgccggg caaacaggca ccgacgtgca gtcgagcgct 960
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<210> 13
<211> 1729

<400> 13 tccacttcgc tgattttgaa attggcagat tcatagaaac gttcaggtgt ggaaatcagg 60 ctgagtgcgc agatttcgtt gataagggtg tggtactggt cattgttggt catttcaagg 120 cetetgagtg eggtgeggag caataceagt etteetgetg gegtgtgeac aetgagtege 180 aggcataggc atttcagttc cttgcgttgg ttgggcatat aaaaaaagga acttttaaaa 240 acagtgcaat gagatgccgg caaaacggga accggtcgct gcgctttgcc actoacttcg 300 agcaagetea accecaaaca tecacatece tategaacgg acagegatac ggccaettge 360 tetggtaaac cetggagetg gegteggtec aattgeecac ttagegaggt aacgeageat 420 gagcategge ateacacece ggcegeaaca gaccaceaeg ceaetegatt trteggeget 480 aageggeaag agteeteaac caaacaegtt eggegageag aacaeteage aagegatega 540 cccgagtgca ctgttgttcg gcagcgacac acagaaagac gtcaacttcg gcacgcccga 600 cagcaccgtc cagaatccgc aggacgccag caagcccaac gacagccagt ccaacatcgc 660 taaattgatc agtgcattga tcatgtcgtt gctgcagatg ctcaccaact ccaataaaaa 720 graggarace astraggase agertgatag craggetert ttreagases arggregget 780 cggtacaceg teggeegata gegggggegg eggtacaceg gatgegacag gtggeggegg 840 cggtgatacg ccaagegeaa caggeggtgg eggeggtgat acteegaceg caacaggegg 900 tggcggcagc ggtggcggcg gcacacccac tgcaacaggt ggcggcagcg gtggcacacc 960 cactgcaaca ggeggtggeg agggtggegt aacacegeaa atcacteege agttggeeaa 1020 ccctaaccgt acctcaggta ctggctcggt gtcggacacc gcaggttcta ccgagcaagc 1080 cggcaagatc aatgtggtga aagacaccat caaggtegge getggegaag tetttgaegg 1140 ccacggcgca accttcactg ccgacaaatc tatgggtaac ggagaccagg gcgaaaatca 1200 gaagcccatg ttcgagctgg ctgaaggcgc tacgttgaag aatgtgaacc tgggtgagaa 1260 cgaggtcgat ggcatccacg tgaaagccaa aaacgctcag gaagtcacca ttgacaacgt 1320 gratgercag aacgteggtg aagacetgat taeggteaaa ggegagggag gegeageggt 1380 cactaatctg aacatcaaga acagcagtgc caaaggtgca gacgacaagg ttgtccagct 1440 caacgccaac acteacttga aaatcgacaa cttcaaggcc gacgatttcg gcacgatggt 1500 togoaccaac ggtggcaago agtttgatga catgagcato gagotgaacg gcatogaago 1560 taaccacggc aagttcgccc tggtgaaaag cgacagtgac gatctgaagc tggcaacggg 1620 caacategee atgacegaeg teaaacaege etaegataaa acceaggeat egacecaaea 1680 1729 caccgagett tgaatecaga caagtagett gaaaaaaggg ggtggaete

<210> 14

<212> DNA

<213> Pseudomonas syringae

<211> 424

<212> PRT

<213> Pseudomonas syringae

-4	n	٥>	14

Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu

1 5 10 15

- Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly 20 25 30
- . Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
 35 40 45
 - Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val 50 55 60
 - Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile 65 70 75 80
 - Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr 85 90 95
 - Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln 100 105 110
 - Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser 115 120 125
 - Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Asp Thr 130 135 140
 - Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly 145 150 155 160
 - Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly 165 170 175
 - Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Gly Gly Gly Val Thr 180 185 190
 - Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr 195 200 205
 - Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile 210 215 220
 - Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp 225 230 235 240
 - Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp 245 250 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr 260 265 270

- Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
 275 280 285
- Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
 290 295 300
- Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala 305 310 315 320
- Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp 325 330 335
- Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
- Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln 355 360 365
- Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly 370 375 380
- Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Leu Lys Leu Ala Thr 385 390 395 400
- Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln 405 410 415
- Ala Ser Thr Gln His Thr Glu Leu
 420

<210> 15

<211> 344

<212> PRT

<213> Pseudomonas solanacearum

<400> 15

- Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
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- Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser 20 25 30
- Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile

- Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
 50 55 60
- Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala

 65 70 75 80
- Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser 85 90 95
- Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
- Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala 115 120 125 .
- Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val 130 135 140
- Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala 145 150 155 160
- Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly 165 170 175
- Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
 180 185 190
- Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala 195 200 205
- Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn 210 220
- Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp 225 230 235 240
- Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn 245 250 255
- Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Asn Gln 260 265 270
- Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly 275 280 285
- Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser

290 295 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val 305 310 315 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln 325 330 335

Gln Ser Thr Ser Thr Gln Pro Met

<210> 16 <211> 1035 <212> DNA <213> Pseudomonas solanacearum

<400> 16 atgtcagtcg gaaacatcca gagcccgtcg aacctcccgg gtctgcagaa cctgaacctc 60 aacaccaaca ccaacagcca gcaatcgggc cagtccgtgc aagacctgat caagcaggtc 120 gagaaggaca teeteaacat categeagee etegtgeaga aggeegeaca gteggeggge 180 ggcascaccg gtaacaccgg caacgcgccg gcgasggacg gcaatgccaa cgcgggcgcc 240 aacgaccega gcaagaacga ceegagcaag agccaggete egcagtegge caacaagace 300 ggcaacgtcg acgacgccaa caaccaggat ccgatgcaag cgctgatgca gctgctggaa 360 gacctggtga agctgctgaa ggcggccctg cacatgcagc agcccggcgg caatgacaag 420 ggcaacggcg tgggcggtgc caacggcgcc aagggtgccg gcggccaggg cggcctggcc 480 gaagegetge aggagatega geagateete geeeageteg geggeggegg tgetggegee 540 ggcggcgcgg gtggcggtgt cggcggtgct ggtggcgcgg atggcggctc cggtgcgggt 600 ggegeaggeg gtgegaacgg cgccgacggc ggcaatggcg tgaacggcaa ccaggcgaac 660 ggcccgcaga acgcaggcga tgtcaacggt gccaacggcg cggatgacgg cagcgaagac 720 cagggeggec teaceggegt getgeaaaag etgatgaaga teetgaaege getggtgeag 780 atgatgcage aaggeggeet eggeggegge aaccaggege agggeggete gaagggtgee 840 ggcaacgest egeoggette eggegegaac eegggegega accageeegg tteggeggat 900 gatcaatcgt ccggccagaa caatctgcaa tcccagatca tggatgtggt gaaggaggtc 960 gtccagatcc tgcagcagat gctggcggcg cagaacggcg gcagccagca gtccacctcg 1020 1035 acgcagccga tgtaa

<210> 17
<211> 26
<212> PRT
<213> Xanthomonas campestris pv. glycines

Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala

1 5 10 15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
20
25

<210> 18
<211> 20
<212> PRT
<213> Xanthomonas campestris pv. pelargonii
<400> 18
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Leu Asp Gln

Leu Leu Ala Met